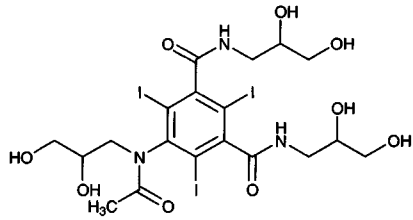
KEY WORDS
plasma; rat; human

REFERENCE
Arbughi,T.; Bertani,F.; Celeste,R.; Grotti,A.; Tirone,P. High-performance liquid chromatographic determination of the X-ray imaging contrast agent, iofratol, in plasma and urine, *J.Chromatogr.B*, **1997**, *701*, 103–113.

Iohexol

Molecular formula: $C_{19}H_{26}I_3N_3O_9$
Molecular weight: 821.14
CAS Registry No.: 66108-95-0
Merck Index: 5068



SAMPLE
Matrix: blood
Sample preparation: Mix serum with an equal volume of MeCN, vortex for 15 s, centrifuge at 14000 g for 30 s, dilute the supernatant 100-fold with mobile phase, inject a 10 µL aliquot.

HPLC VARIABLES
Column: 40 × 3.2 3 µm Velosep RP-18 (Applied Biosystems)
Mobile phase: 8 mM pH 2 Phosphoric acid
Flow rate: 1
Injection volume: 10
Detector: UV 254

KEY WORDS
serum

REFERENCE
Shihabi,Z.K.; Constantinescu,M.S. Iohexol in serum determined by capillary electrophoresis, *Clin.Chem.*, **1992**, *38*, 2117–2120.

SAMPLE
Matrix: blood
Sample preparation: 50 µL Serum + 50 µL 250 µg/mL acetaminophen in 100 mM HCl, add to SPE cartridge containing 150 mg 80-100 mesh Chromosorb P/NAW, elute with 1 mL ethyl acetate:MeOH 5:1, add the eluate to 50 µL 100 mM HCl, vortex for 15 s, centrifuge at 10000 g for 3 min, inject a 20 µL aliquot of the lower aqueous phase.

HPLC VARIABLES
Column: 5 µm C8
Mobile phase: MeCN:20 mM pH 3.3 phosphoric acid 2.5:97.5
Injection volume: 20
Detector: UV 254

CHROMATOGRAM
Internal standard: acetaminophen
Limit of detection: <1 µg/mL

OTHER SUBSTANCES
Extracted: aminohippuric acid (PAH)

KEY WORDS
serum; SPE

REFERENCE
Andreeva,M.; Rapondjieva,A.; Deskova,D.; Tishkov,I.; Svinarov,D. Liquid chromatographic determination of iohexol and PAH with Chromosorb P column used for sample preparation (Abstract 175), *Ther.Drug Monit.*, **1995**, *17*, 427.

SAMPLE
Matrix: blood, tissue
Sample preparation: Plasma. 50 μL Plasma + 150 μL buffer, centrifuge at 1000 g at 0° for 10 min, inject a 10 μL aliquot. Tissue. Weigh 2 testes, add 5 mL buffer, homogenize (Kinematica type PT 10/35, setting 7.5) for 1 min, vortex, centrifuge at 1000 g at 0° for 10 min. Remove a 50 μL aliquot of the supernatant and add it to 150 μL buffer, centrifuge at 1000 g at 0° for 10 min, inject a 10 μL aliquot. (Buffer was 5 mM metaphosphoric acid and 5 mM disodium EDTA.)

HPLC VARIABLES
Guard column: C18 Bondapak guard column
Column: 300 × 3.9 10 μm μBondapak C18
Mobile phase: MeCN:buffer 2.5:97.5–5:95 (Buffer was 100 mM NaH_2PO_4 and 0.2 mM Na_2EDTA adjusted to pH 3.1 with orthophosphoric acid.)
Flow rate: 1
Injection volume: 10
Detector: UV 254

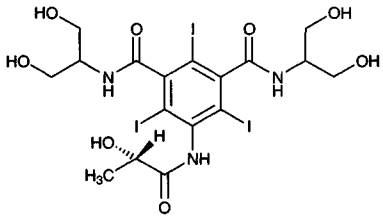
CHROMATOGRAM
Retention time: 7.5 (two isomers seen)
Limit of detection: 670 ng/mL

OTHER SUBSTANCES
Also analyzed: iopamidol, diatrizoate

KEY WORDS
plasma; mouse; testes

REFERENCE
Harapanhalli,R.S.; Yaghmai,V.; Patel,Y.D.; Baker,S.R.; Rao,D.V. Assay of radiographic contrast agents in mice plasma and testes by high-performance liquid chromatography, *Anal.Chem.*, **1993**, *65*, 606–612.

Iopamidol



Molecular formula: $C_{17}H_{22}I_3N_3O_8$
Molecular weight: 777.09
CAS Registry No.: 60166-93-0
Merck Index: 5071

SAMPLE
Matrix: blood, tissue
Sample preparation: Plasma. 50 μL Plasma + 150 μL buffer, centrifuge at 1000 g at 0° for 10 min, inject a 10 μL aliquot. Tissue. Weigh 2 testes, add 5 mL buffer, homogenize (Kinematica type PT 10/35, setting 7.5) for 1 min, vortex, centrifuge at 1000 g at 0° for 10 min. Remove a 50 μL aliquot of the supernatant and add it to 150 μL buffer, centrifuge at 1000 g at 0° for 10 min, inject a 10 μL aliquot. (Buffer was 5 mM metaphosphoric acid and 5 mM disodium EDTA.)

HPLC VARIABLES
Guard column: C18 Bondapak guard column
Column: 300 × 3.9 10 μm μBondapak C18
Mobile phase: MeCN:buffer 2.5:97.5–5:95 (Buffer was 100 mM NaH_2PO_4 and 0.2 mM Na_2EDTA adjusted to pH 3.1 with orthophosphoric acid.)

Flow rate: 1
Injection volume: 10
Detector: UV 254

CHROMATOGRAM
Retention time: 9.5
Limit of detection: 640 ng/mL

OTHER SUBSTANCES
Also analyzed: iohexaol, diatrizoate

KEY WORDS
plasma; mouse; testes

REFERENCE
Harapanhalli,R.S.; Yaghmai,V.; Patel,Y.D.; Baker,S.R.; Rao,D.V. Assay of radiographic contrast agents in mice plasma and testes by high-performance liquid chromatography, *Anal.Chem.*, **1993**, *65*, 606–612.

SAMPLE
Matrix: blood, urine
Sample preparation: Plasma. Add 30 μL water to 100 μL plasma. Add 30 μL 35% perchloric acid. Agitate and centrifuge at 3500 g for 10 min. Inject a 10 μL aliquot of the clear supernatant. Urine. Dilute 1 mL urine with 2 mL water, centrifuge at 4500 g for 15 min. Add 100 μL 5 mg/mL IS, 100 μL glacial acetic acid, and an ion-exchange resin mixture (1 g Duolite A-30B + 900 mg Amberlite IR-120). Dilute the suspension to 5 mL with water. Agitate for 30 min and centrifuge at 3500 g for 5 min. Inject a 10 μL aliquot of the supernatant.

HPLC VARIABLES
Guard column: 30 × 4.0 7 μm LiChrosorb RP-8
Column: 250 × 4.6 5 μm LiChrosorb RP-8
Mobile phase: MeCN:5 mM pH 4.5 potassium dihydrogen phosphate 5:95
Column temperature: 45
Flow rate: 1.0
Injection volume: 10
Detector: UV 242

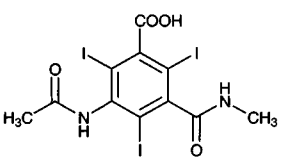
CHROMATOGRAM
Retention time: 4.1

OTHER SUBSTANCES
Extracted: iofratol

KEY WORDS
iopamidol is IS; plasma; rat; human

REFERENCE
Arbughi,T.; Bertani,F.; Celeste,R.; Grotti,A.; Tirone,P. High-performance liquid chromatographic determination of the X-ray imaging contrast agent, iofratol, in plasma and urine, *J.Chromatogr.B*, **1997**, *701*, 103–113.

Iothalamate



Molecular formula: $C_{11}H_9I_3N_2O_4.C_7H_{17}NO_5$ (meglumine), $C_{11}H_8I_3N_2NaO_4$ (sodium salt)
Molecular weight: 809.13 (meglumine), 635.90 (sodium salt)
CAS Registry No.: 13087-53-1, 2276-90-6 (iothalamic acid), 6284-40-8 (meglumine), 17692-74-9 (^{125}I radioactive agent), 15845-98-4 (^{131}I radioactive agent), 1225-20-3 (sodium salt)

SAMPLE
Matrix: blood, urine
Sample preparation: Dilute urine 10-fold with water. Add 200 μL MeCN containing 20 μg/mL p-aminobenzoic acid to 100 μL diluted urine, vortex briefly, centrifuge at 12000 g for 4 min, inject a 20 μL aliquot of the supernatant.

HPLC VARIABLES
Guard column: 5 μm Ultrasphere C18
Column: 250 mm 5 μm Primesphere C18 (Torrance, USA)
Mobile phase: MeOH:buffer 18:82 (Buffer was 50 mM NaH_2PO_4 with 0.5 mM tetrabutyl ammonium hydrogen sulfate with an unadjusted pH of 4.11.)
Flow rate: 0.8
Injection volume: 20
Detector: UV 254

CHROMATOGRAM
Retention time: 13
Internal standard: p-aminobenzoic acid (9.8)

OTHER SUBSTANCES
Extracted: p-aminohippuric acid

KEY WORDS
serum

REFERENCE
Agarwal,R. Chromatographic estimation of iothalamate and p-aminohippuric acid to measure glomerular filtration rate and effective renal plasma flow in humans, *J.Chromatogr.B*, **1998**, *705*, 3–9.

SAMPLE
Matrix: formulations
Sample preparation: Mix a dilution of the injectable solution in water with a mixture of 2,4-dinitrobenzenesulfonyl chloride (DNBS-Cl) and sodium carbonate in acetone at ambient temperature for 1 hr. Dilute the reaction mixture with 25% MeOH and inject a 20 μL aliquot.

HPLC VARIABLES
Column: 150 × 4.6 4 μm Nova-Pak C18
Mobile phase: MeOH:buffer 25:75 (Buffer was 20 mM tetrapropylammonium hydroxide adjusted to pH 3.4 with orthophosphoric acid.)
Flow rate: 1.3
Injection volume: 20
Detector: UV 262

CHROMATOGRAM
Retention time: 2.75
Limit of quantitation: 1 μg

OTHER SUBSTANCES
Simultaneous: diatrizoate, meglumine

KEY WORDS
injections; only meglumine is derivatized under these conditions

REFERENCE
Lau-Cam,C.A.; Roos,R.W. HPLC method with spectrophotometric detection for the simultaneous assay of meglumine and its counterions iothalamic acid or diatrizoic acid in radiographic solutions for injection (Abstract 3372), *Pharm.Res.*, **1997**, *14*, S586.

SAMPLE
Matrix: blood
Sample preparation: 100 μL Plasma or urine + 0.5-5 μg p-aminobenzoic acid + 200 μL MeCN, vortex for a few s, centrifuge at 800 g for 5 min, inject a 5 μL aliquot of the supernatant.

HPLC VARIABLES
Column: 300 × 3.9 10 μm μBondapak C18
Mobile phase: MeCN:0.04% pH 2.5 ± 0.05 phosphoric acid 3.5:96.5
Flow rate: 1.5
Injection volume: 5
Detector: UV 254

CHROMATOGRAM
Retention time: 6
Internal standard: p-aminobenzoic acid (8)
Limit of detection: 500 ng/mL

OTHER SUBSTANCES
Extracted: p-aminohippuric acid

KEY WORDS
plasma; dog; human; pharmacokinetics

REFERENCE
Prueksaritanont,T.; Chen,M.L.; Chiou,W.L. Simple and micro high-performance liquid chromatographic method for simultaneous determination of p-aminohippuric acid and iothalamate in biological fluids, *J.Chromatogr.*, **1984**, *306*, 89–97.

SAMPLE
Matrix: blood
Sample preparation: Add barbital to plasma. 100 μL Plasma + 500 μL MeOH, vortex for 15 s, centrifuge at 2500 rpm for 10 min. Remove the organic layer and evaporate it to dryness under a stream of nitrogen, reconstitute the residue in buffer, inject a 15-20 μL aliquot.

HPLC VARIABLES
Column: 250 × 4.6 5 μm Octyl C8 (Rainin)
Mobile phase: MeOH:MeCN:buffer 90:10:300 (Buffer was 6.44 g KH_2PO_4, 7.04 g K_2HPO_4, and 14 mL 500 mM dodecyltriethylammonium phosphate (Regis) in 4 L water.)
Flow rate: 1
Injection volume: 15-20
Detector: UV 254

CHROMATOGRAM
Retention time: 18.2
Internal standard: barbital (15.9)
Limit of quantitation: 3000 ng/mL

OTHER SUBSTANCES
Extracted: p-aminohippuric acid

KEY WORDS
plasma

REFERENCE
Jayewardene,A.L.; Seneviratne,A.K.; Gambertoglio,J.G. Paired ion reversed-phase HPLC assay for the simultaneous determination of iothalamic acid and para aminohippuric acid in plasma, *J.Liq.Chromatogr.*, **1994**, *17*, 2395–2412.

SAMPLE
Matrix: blood, urine
Sample preparation: Plasma. 0.5-1 mL Plasma + 18 μg iodamide + 2 μg hippuric acid + 1 mL 1 M HCl + 5 mL ethyl acetate, shake for 10 min, centrifuge, repeat extraction. Combine the organic layers and evaporate them under a stream of nitrogen at 60°, add 3 mL 100 mM NaOH, shake for 10 min, centrifuge, discard the organic layer. Remove the aqueous layer and add it to 500 μL 1 M HCl, add 5 mL ethyl acetate, shake for 10 min, centrifuge, repeat extraction. Combine the organic layers and evaporate them to dryness under a stream of nitrogen at 60°, reconstitute the residue in 100 μL mobile phase, inject a 10-20 μL aliquot. Urine. 1 mL Urine + 10 μL 10% iodamide in water, mix. Remove a 100 μL aliquot and add it to 1 mL 1 M HCl, add 5 mL ethyl acetate, shake for 10 min, centrifuge, repeat extraction. Combine the organic layers and evaporate them to dryness under a stream of nitrogen at 60°, reconstitute the residue in 400 μL mobile phase, inject a 10-20 μL aliquot.

HPLC VARIABLES
Column: 250 × 2.6 10 μm ODS-HC Sil-X-1 (Perkin-Elmer)
Mobile phase: MeCN:water:85% phosphoric acid 4:96:0.03
Flow rate: 1
Injection volume: 10-20
Detector: UV 235

CHROMATOGRAM
Retention time: 2.0
Internal standard: iodamide (2.5), hippuric acid (5.6)
Limit of detection: 500 ng/mL

OTHER SUBSTANCES
Extracted: o-iodohippurate

KEY WORDS
plasma; pharmacokinetics

REFERENCE
Boschi,S.; Marchesini,B. High-performance liquid chromatographic method for the simultaneous determination of iothalamate and o-iodohippurate, *J.Chromatogr.*, **1981**, *224*, 139–143.

SAMPLE
Matrix: blood, urine
Sample preparation: 200 μL Plasma + 200 μL 100 mM NaH_2PO_4 + 400 μL MeCN, mix for 5 s, let stand at 4° for 15 min, centrifuge at 10500 g for 1 min. Remove the supernatant and add it to 2 mL dichloromethane, mix for 5 min, centrifuge at 4800 g for 10 min, inject a 5-50 μL aliquot of the upper aqueous phase. Urine. Centrifuge urine at 4800 g for 10 min, dilute 1:10 with 50 mM NaH_2PO_4, inject an aliquot.

HPLC VARIABLES
Column: 125 × 4 5 μm LiChrosorb RP-18
Mobile phase: MeCN:water 7.5:92.5 containing 5.50 g/L $NaH_2PO_4.H_2O$, 1.80 g/L $Na_2HPO_4.2H_2O$, and 20 mg/L tetrabutylammonium bromide, pH 6.4 (plasma) or MeCN:water 5:95 containing 5.50 g/L $NaH_2PO_4.H_2O$, 1.80 g/L $Na_2HPO_4.2H_2O$, and 22.5 mg/L tetrabutylammonium bromide, pH 6.4 (urine)
Flow rate: 1
Injection volume: 5-50
Detector: UV 254

CHROMATOGRAM
Retention time: 2.3 (plasma), 2.7 (urine)
Limit of detection: 1000 ng/mL

OTHER SUBSTANCES
Extracted: cefotetan (UV 280)

KEY WORDS
plasma; pharmacokinetics

REFERENCE
Kees,F.; Grobecker,H.; Naber,K.G. High-performance liquid chromatographic analysis of cefotetan epimers in human plasma and urine, *J.Chromatogr.*, **1984**, *305*, 363–371.

SAMPLE
Matrix: blood, urine
Sample preparation: Plasma. 500 μL Plasma + 500 μL 1 M HCl, vortex for 10 s, add 6 mL ethyl acetate, vortex for 20 s, centrifuge at 4° at 1700 g for 10 min. Remove the organic layer and evaporate it to dryness under a stream of nitrogen, reconstitute the residue in 400 μL 25 mM pH 3 KH_2PO_4, add 500 μL dichloromethane, shake gently horizontally for 5 min, centrifuge at 1700 g for 5 min, inject a 20 μL aliquot of the aqueous phase. Urine. Dilute 1:10 with water. Remove a 1 mL aliquot and add it to 1 mL 1 M HCl, vortex for 10 s, add 6 mL ethyl acetate, vortex for 20 s, centrifuge at 4° at 1700 g for 10 min. Remove the organic layer and evaporate it to dryness under a stream of nitrogen, reconstitute the residue in 400 μL 25 mM pH 3 KH_2PO_4, add 500 μL dichloromethane, shake gently horizontally for 5 min, centrifuge at 1700 g for 5 min, inject a 20 μL aliquot of the aqueous phase.

HPLC VARIABLES
Guard column: 10 × 3 anion-exchange guard column (Chrompack)
Column: 250 × 4.6 Partisil 10 SAX
Mobile phase: MeCN:25 mM pH 3 phosphate buffer 15:85
Flow rate: 1
Injection volume: 20
Detector: UV 254

CHROMATOGRAM
Retention time: 7.05
Limit of detection: 500 ng/mL

OTHER SUBSTANCES
Extracted: p-aminohippuric acid
Noninterfering: acipimox, allopurinol, aspirin, atenolol, captopril, chlorthalidone, clonidine, digitoxin, digoxin, diltiazem, dipyridamole, enalapril, furosemide, gemfibrozil, hydralazine, hydrochlorothiazide, ibopamine, insulin, inulin, isosorbide dinitrate, α-methyldopa, nicardipine, nifedipine, prazosin, propranolol, salicylic acid, simvastatin, trinitrin, verapamil

KEY WORDS
plasma; pharmacokinetics

REFERENCE
Gaspari,F.; Mainardi,L.; Ruggenenti,P.; Remuzzi,G. High-performance liquid chromatographic determination of iothalamic acid in human plasma and urine, *J.Chromatogr.*, **1991**, *570*, 435–440.

SAMPLE
Matrix: urine
Sample preparation: Dilute urine 1:100 or 1:500. 200 μL Diluted urine + 50 μL barbital solution, vortex for 15 s, inject a 20-30 μL aliquot.

HPLC VARIABLES
Column: 250 × 4.6 5 μm Ultrasphere C8
Mobile phase: MeCN:MeOH:10 mM pH 7.5 potassium phosphate buffer:0.5 M dodecyl triethylammonium phosphate 6:94:300:0.6 (0.5 M Dodecyl triethylammonium phosphate was Q-12, Ion pair reagent, Regis Chemical Co.)
Flow rate: 1
Injection volume: 20-30

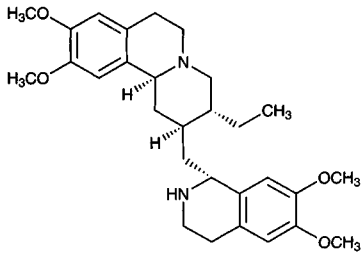
Detector: UV 254

CHROMATOGRAM
Retention time: 16.8
Internal standard: barbital (14.5)
Limit of quantitation: 50000 ng/mL

OTHER SUBSTANCES
Extracted: p-aminohippuric acid

REFERENCE
Seneviratne,A.K.; Jayewardene,A.L.; Gambertoglio,J.G. Paired-ion reversed-phase HPLC assay for the determination of iothalamic acid and para aminohippuric acid in urine, *J.Pharm.Biomed.Anal.*, **1994**, *12*, 1311–1316.

Ipecac



Molecular formula: $C_{28}H_{38}N_2O_4$, $C_{29}H_{40}N_2O_4$
Molecular weight: 466.62, 480.65
CAS Registry No.: 483-17-0, 483-18-1
Merck Index: 5086

SAMPLE
Matrix: blood, emesis, urine
Sample preparation: 2 mL Plasma, whole blood, urine, or emesis (diluted 1:100) + 100 μL 10 ng/mL N-propylprocainamide in water + 2 mL saturated sodium borate + 7 mL n-butyl chloride, vortex for 30 s, centrifuge. Remove the organic phase and add it to 200 μL 10 mM HCl, vortex, centrifuge, inject a 30-50 μL aliquot of the aqueous phase.

HPLC VARIABLES
Column: 150 × 4.6 5 μm Ultrasphere ODS
Mobile phase: MeOH:25 mM pH 8.0 Na_2HPO_4 72:28
Flow rate: 1.7
Injection volume: 30-50
Detector: F ex 285 em 316

CHROMATOGRAM
Retention time: 3 (cephaeline), 4.1 (emetine)
Internal standard: N-propylprocainamide (1.7)
Limit of quantitation: 5 ng/mL

KEY WORDS
plasma; whole blood

REFERENCE
Crouch,D.J.; Moran,D.M.; Finkle,B.S.; Peat,M.A. Quantitative analysis of emetine and cephaeline by reversed-phase high performance liquid chromatography with fluorescence detection, *J.Anal.Toxicol.*, **1984**, *8*, 63–65.

SAMPLE
Matrix: blood, vomit
Sample preparation: Mix 250 μL vomit or 500 μL serum with an equal volume of 10% ammonium hydroxide for 5 min, add 4 mL ether, mix. Remove the organic layer and evaporate it

to dryness under vacuum at 25°, reconstitute the residue in 10 (vomit) or 0.2 (serum) mL 0.01% HCl. Mix 1 mL vomit solution with 50 μL 4 mg/mL acrinol or 200 μL serum solution with 50 μL 200 μg/mL acrinol, inject a 100 μL aliquot.

HPLC VARIABLES
Column: 150 × 4.6 5 μm TSK gel ODS-80TM
Mobile phase: MeOH:buffer 54:46 (Buffer was 10 mM sodium 1-heptanesulfonate adjusted to pH 4 with glacial acetic acid.)
Flow rate: 1
Injection volume: 100
Detector: F ex 285 em 316

CHROMATOGRAM
Internal standard: acrinol

KEY WORDS
dog; serum; pharmacokinetics; assay determines cephaeline, a constituent of ipecac

REFERENCE
Teshima,D.; Suzuki,A.; Otsubo,K.; Higuchi,S.; Aoyama,T.; Shimozono,Y.; Saita,M.; Noda,K. Efficacy of emetic and United State Pharmacopoeia ipecac syrup in prevention of drug absorption, *Chem.Pharm.Bull.(Tokyo)*, **1990**, *38*, 2242–2245.

SAMPLE
Matrix: cell cultures
Sample preparation: Dry 1-5 g cell culture at 60°, powder, extract with MeOH in a glass percolator for 72 h. Filter (paper) extract, wash solid with MeOH at 45°, concentrate filtrate under reduced pressure, filter (0.5 μm), dilute filtrate with MeOH, inject a 2.5 μL aliquot.

HPLC VARIABLES
Column: 250 × 4 10 μm silica gel (Spectra Physics)
Mobile phase: Chloroform:MeOH:diethylamine 90:10:0.2
Flow rate: 0.5
Injection volume: 2.5
Detector: UV 280

CHROMATOGRAM
Retention time: 4.5 (emetine), 5 (cephaeline)

KEY WORDS
normal phase

REFERENCE
Jha,S.; Sahu,N.P.; Mahato,S.B. Production of the alkaloids emetine and cephaeline in callus cultures of *Cephaelis ipecacuanha*, *Planta Med.*, **1988**, *54*, 504–506.

SAMPLE
Matrix: formulations
Sample preparation: Syrup. Dilute syrup with an equal volume of water. 2 mL Diluted syrup + 2 mL 1% dansyl chloride in acetone + 200 μL 1.5 M sodium carbonate, heat at 45 ± 2° in the dark for 20 min, cool, add 3 mL water, add 500 μL benzene (Caution! Benzene is a carcinogen!), shake. Remove the organic layer and evaporate it to dryness, reconstitute the residue in mobile phase, inject a 10 μL aliquot. Capsules. Sonicate the contents of a capsule in 20 mL water for 10 min, centrifuge at 2000 g for 10 min. 2 mL Supernatant + 2 mL 1% dansyl chloride in acetone + 200 μL 1.5 M sodium carbonate, heat at 45 ± 2° in the dark for 20 min, cool, add 3 mL water, add 500 μL benzene (Caution! Benzene is a carcinogen!), shake. Remove the organic layer and evaporate it to dryness, reconstitute the residue in mobile phase, inject a 10 μL aliquot.

HPLC VARIABLES
Column: 250 × 2.8 10 μm silica gel SI 100 (Merck)

Mobile phase: Diisopropyl ether:isopropanol:concentrated ammonia 48:2:0.3 (Caution! Diisopropyl ether readily forms explosive peroxides!)
Injection volume: 10
Detector: UV 254 or F ex 358 em 492 (cephaeline) or ex 356 em 481 (emetine)

CHROMATOGRAM
Retention time: 2.5 (cephaeline), 3.5 (emetine)

OTHER SUBSTANCES
Simultaneous: ephedrine, codeine (not derivatized, detect at UV 254 only)

KEY WORDS
derivatization; syrup; capsules; normal phase

REFERENCE
Frei,R.W.; Santi,W.; Thomas,M. Liquid chromatography of dansyl derivatives of some alkaloids and the application to the analysis of pharmaceuticals, *J.Chromatogr.*, **1976**, *116*, 365–377.

SAMPLE
Matrix: formulations
Sample preparation: Mix 10 g linctus with 10-20 mL 200 μg/mL ethyl 4-hydroxybenzoate in MeCN:mobile phase 10:90, make up to 100 mL with mobile phase, inject a 10-25 μL aliquot. Mix 10 pastilles with 50 mL 200 μg/mL ethyl 4-hydroxybenzoate in MeCN:mobile phase 10:90, make up to 200 mL with mobile phase, inject a 10-25 μL aliquot.

HPLC VARIABLES
Column: 150 × 3.9 μBondapak C18
Mobile phase: MeOH:water 40:60 containing 1 g/L sodium 1-heptanesulfonate and 1 mL/L orthophosphoric acid
Column temperature: 35
Flow rate: 2
Injection volume: 10-25
Detector: F ex 276 em 304

CHROMATOGRAM
Retention time: 8 (cephaeline), 15 (emetine)
Internal standard: ethyl 4-hydroxybenzoate (5)
Limit of quantitation: 5 μg/g

OTHER SUBSTANCES
Simultaneous: dihydroemetine (UV 214), emetamine (UV 214), O-methylpsychotrine (UV 214), tetrahydroemetine (UV 214)

KEY WORDS
stability-indicating; linctus; pastilles; rugged

REFERENCE
Elvidge,D.A.; Johnson,G.W.; Harrison,J.R. Selective, stability-indicating assay of the major ipecacuanha alkaloids, emetine and cephaeline, in pharmaceutical preparations by high-performance liquid chromatography using spectrofluorimetric detection, *J.Chromatogr.*, **1989**, *463*, 107–118.

SAMPLE
Matrix: formulations
Sample preparation: 2 mL Sample + 1 mL 200 μg/mL emetine hydrochloride in water, make up to 10 mL with mobile phase, filter (0.45 μm), inject a 50-100 μL aliquot.

HPLC VARIABLES
Column: 100 × 4.6 5 μm Technosphere RP C-8 (HPLC Technology)
Mobile phase: MeCN:40 mM tetramethylammonium bromide:1 M acetic acid 80:15:5 (apparent pH 4.5)

Flow rate: 1.5
Injection volume: 50-100
Detector: UV 260

CHROMATOGRAM
Retention time: 1.83
Internal standard: emetine

OTHER SUBSTANCES
Simultaneous: benzalkonium (C12, C14, C16), tetrahydrozoline, naphazoline

KEY WORDS
nasal; ophthalmic; emetine is IS

REFERENCE
Santoni,G.; Medica,A.; Gratteri,P.; Furlanetto,S.; Pinzauti,S. High-performance liquid chromatographic determination of benzalkonium and naphazoline or tetrahydrozoline in nasal and ophthalmic solutions, *Farmaco*, **1994**, *49*, 751–754.

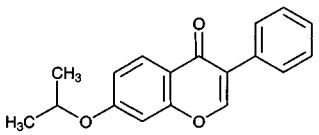
SAMPLE
Matrix: solutions

HPLC VARIABLES
Column: 300 × 4 C18 Micropak SP
Mobile phase: MeCN:3 mM pH 2.5 phosphoric acid 20:80
Flow rate: 2
Injection volume: 15
Detector: F ex 285 em 315 or UV 205

CHROMATOGRAM
Retention time: 4.1 (cephaeline), 6.2 (emetine)
Limit of detection: 30 ng/mL

REFERENCE
Lachman,M.F.; Romeo,R.; McComb,R.B. Emetine identified in urine by HPLC, with fluorescence and ultraviolet/diode array detection, in a patient with cardiomyopathy, *Clin.Chem.*, **1989**, *35*, 499–502.

Ipriflavone



Molecular formula: $C_{18}H_{16}O_3$
Molecular weight: 280.32
CAS Registry No.: 35212-22-7
Merck Index: 5090

SAMPLE
Matrix: bulk
Sample preparation: Inject a 10 μL aliquot of a solution in MeOH.

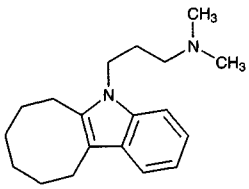
HPLC VARIABLES
Column: 30 × 4.6 3 μm ODS C18 (Perkin-Elmer)
Mobile phase: MeCN:buffer 50:50 (Buffer was 10 mM triethylamine adjusted to pH 2.5 with orthophosphoric acid.)
Flow rate: 1.2
Injection volume: 10
Detector: UV 225

CHROMATOGRAM
Retention time: 3.4

OTHER SUBSTANCES
Simultaneous: impurities

REFERENCE
Sustacha,K.; Chacón,M.; Lucero,M.L.; Orjales,A. Determination of ipriflavone and its synthetic impurities by high-performance liquid chromatography using diode-array detection, *J.Chromatogr.A*, **1996**, *719*, 245–250.

Iprindole



Molecular formula: $C_{19}H_{28}N_2$
Molecular weight: 284.44
CAS Registry No.: 5560-72-5, 20432-64-8 (HCl)
Merck Index: 5091
Lednicer No.: 1 318

SAMPLE
Matrix: solutions
Sample preparation: Prepare a 10 μg/mL solution in MeOH, inject a 20 μL aliquot.

HPLC VARIABLES
Column: 125 × 4.9 Spherisorb S5W silica
Mobile phase: MeOH containing 10 mM ammonium perchlorate and 1 mL/L 100 mM NaOH in MeOH, pH 6.7
Flow rate: 2
Injection volume: 20
Detector: E, LeCarbone, V25 glassy carbon electrode, + 1.2 V

CHROMATOGRAM
Retention time: 4.5

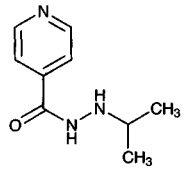
OTHER SUBSTANCES
Also analyzed: acebutolol, acepromazine, acetophenazine, N-acetylprocainamide, albuterol, alprenolol, amethocaine, amiodarone, amitriptyline, antazoline, atenolol, azacyclonal, bamethan, benactyzine, benperidol, benzethidine, benzocaine, benzoctamine, benzphetamine, benzquinamide, bromhexine, bromodiphenhydramine, bromperidol, brompheniramine, brompromazine, buclizine, bufotenine, bupivacaine, buprenorphine, butacaine, butethamate, chlorcyclizine, chlorpheniramine, chlorphenoxamine, chlorprenaline, chlorpromazine, chlorprothixene, cimetidine, cinchonidine, cinnarizine, clemastine, clomipramine, clonidine, cocaine, cyclazocine, cyclizine, cyclopentamine, cyproheptadine, deserpidine, desipramine, dextromoramide, dextropropoxyphene, dicyclomine, diethylcarbamazine, diethylpropion, diethylthiambutene, dihydroergotamine, dimethindene, dimethothiazine, diphenhydramine, diphenoxylate, dipipanone, diprenorphine, dipyridamole, disopyramide, dothiepin, doxapram, doxepin, doxylamine, droperidol, ephedrine, ergocornine, ergocristine, ergocristinine, ergocryptine, ergometrine, ergosine, ergosinine, ergotamine, ethopropazine, etorphine, etoxeridine, fenethazine, fenfluramine, fenoterol, fentanyl, flavoxate, fluopromazine, flupenthixol, fluphenazine, flurazepam, haloperidol, hydroxyzine, hyoscine, ibogaine, imipramine, indapamine, isothipendyl, isoxsuprine, ketanserin, laudanosine, lidocaine, lofepramine, loxapine, maprotiline, mecamylamine, meclophenoxate, meclozine, medazepam, mephentermine, mepivacaine, meptazinol, mepyramine, mesoridazine, metaraminol, methadone, methamphetamine, methapyrilene, methdilazene, methotrimeprazine, methoxamine, methoxyphenamine, methoxypromazine, methylephedrine, methylergonovine, methysergide, metoclopramide, metopimazine, metoprolol, mianserin, morazone, nadolol, nalorphine, naloxone, naphazoline, nicotine, nifedipine, nomifensine, nortriptyline, noscapine, orphenadrine, oxeladin, oxprenolol, oxymetazolin, papaverine, pargyline, pecazine, penbutolol, pentazocine, penthienate, pericyazine, perphenazine, phenadoxone, phenampromide, phenazocine, phenbutrazate, phendimetrazine, phenelzine, phenglutarimide,

phenindamine, pheniramine, phenmetrazine, phenomorphan, phenoperidine, phenothiazine, phenoxybenzamine, phentolamine, phenylephrine, phenyltoloxamine, physostigmine, piminodine, pimozide, pindolol, pipamazine, pipazethate, piperacetazine, piperidolate, pipradol, pirenzepine, piritramide, pizotifen, practolol, pramoxine, prazosin, prenylamine, prilocaine, primaquine, proadifen, procainamide, procaine, prochlorperazine, procyclidine, proheptazine, prolintane, promazine, promethazine, pronethalol, properidine, propiomazine, propranolol, prothipendyl, protriptyline, proxymetacaine, pseudoephedrine, pyrimethamine, quinidine, quinine, ranitidine, rescinnamine, sotalol, tacrine, terazosin, terbutaline, terfenadine, thenyldiamine, theophylline, thiethylperazine, thiopropazate, thioproperazine, thioridazine, thiothixene, thonzylamine, timolol, tocainide, tolpropamine, tolycaine, tranylcypromine, trazodone, trifluoperazine, trifluperidol, trimeperidine, trimeprazine, trimethobenzamide, trimethoprim, trimipramine, tripelennamine, triprolidine, tryptamine, verapamil, xylometazoline

REFERENCE

Jane,I.; McKinnon,A.; Flanagan,R.J. High-performance liquid chromatographic analysis of basic drugs on silica columns using non-aqueous ionic eluents. II. Application of UV, fluorescence and electrochemical oxidation detection, *J.Chromatogr.*, **1985**, *323*, 191–225.

Iproniazid



Molecular formula: $C_9H_{13}N_3O$
Molecular weight: 179.22
CAS Registry No.: 54-92-2, 305-33-9 (phosphate)
Merck Index: 5094
Lednicer No.: 1 254

SAMPLE

Matrix: blood
Sample preparation: 2 mL Whole blood or plasma + 2 mL buffer + 5 mL chloroform:isopropanol: n-heptane 60:14:26, shake gently horizontally for 10 min, centrifuge at 2800 g for 10 min. Remove the lower organic layer and evaporate it to dryness under vacuum at 45°, reconstitute the residue in 100 µL mobile phase, centrifuge at 2800 g for 5 min, inject a 50 µL aliquot of the supernatant. (Buffer was saturated ammonium chloride solution 25% diluted with water, adjusted to pH 9.5 with 25% ammonia solution.)

HPLC VARIABLES

Column: 300 × 3.9 4 µm NovaPack C18
Mobile phase: MeOH:THF:buffer 65:5:30 (Buffer was 0.68 g/L (10 mM (sic)) KH_2PO_4 adjusted to pH 2.6 with concentrated orthophosphoric acid.) (At the end of each session wash the column with water for 1 h and MeOH for 1 h, re-equilibrate for 30 min.)
Column temperature: 30
Flow rate: 0.8
Injection volume: 50
Detector: UV 264

CHROMATOGRAM

Retention time: 3.00
Limit of detection: <120 ng/mL

KEY WORDS

whole blood; plasma; interferences may occur–compounds (all of which are extracted) elute in this order tenoxicam; iproniazid; methocarbamol; methotrexate; caffeine; nialamide; colchicine; cytarabine; benzoylecgonine; acetaminophen; diazoxide; dacarbazine; sulfinpyrazole; flumazenil; sulpride; morphine; atenolol; toloxatone; terbutaline; albuterol; phenobarbital; ranitidine; tiapride; phenol; chlormezanone; aspirin; metformin; ritodrine; codeine; sultopride; amisulpride; naltrexone; lisinopril; benzocaine; nizatidine; nalorphine; mephenesin; naloxone; sotalol; carteolol; procainamide; carbamazepine; bromazepam; nalbuphine; nadolol; procarbazine; dihydralazine; omeprazole; strychnine; acebutolol; glutethimide; chlorpropamide; glipizide; triazolam; prazosin; flunitrazepam; clonazepam; metoclopramide; melphalan; estazolam;

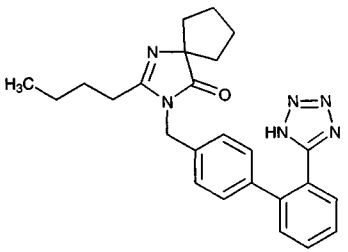
tolbutamide; ephedrine; clonidine; pindolol; clobazam; minoxidil; disopyramide; nitrazepam; dextromethorphan; tofisopam; zopiclone; debrisoquine; sulindac; alprazolam; cycloguanil; lorazepam; methaqualone; ketamine; piroxicam; metoprolol; nifedipine; quinine; mephentermine; prilocaine; pentazocine; oxazepam; tiaprofenic acid; quinidine; celiprolol; ajmaline; yohimbine; lidocaine; secobarbital; viloxazine; mepivacaine; meperidine; doxylamine; labetalol; temazepam; amodiaquine; benperidol; droperidol; hydroxychloroquine; zolpidem; ketoprofen; alminoprofen; cicletanine; moclobemide; chloroquine; cocaine; timolol; nomifensine; ticlopidine; acenocoumarol; vindesine; mexiletine; dipyridamole; trazodone; pipamperone; pyrimethamine; benazepril; vincristine; metapramine; chlordiazepoxide; oxprenolol; warfarin; clorazepate; flecainide; phencyclidine; thiopental; fenfluramine; metipranolol; triprolidine; naproxen; buprenorphine; verapamil; buspirone; tianeptine; midazolam; bupivacaine; carbinoxamine; loprazolam; cetirizine; chlorpheniramine; moperone; cibenzoline; medifoxamine; astemizole; vinblastine; nicardipine; bisoprolol; diltiazem; glibornuride; reserpine; aconitine; nitrendipine; diazepam; mianserin; ramipril; haloperidol; tetracaine; alprenolol; aceprometazine; glibenclamide; chlorophenacinone; doxepin; nimodipine; diphenhydramine; cyclizine; histapyrrodine; phenylbutazone; demexiptiline; clozapine; proguanil; trifluperidol; medazepam; cyamemazine; bumadizone; suriclone; propranolol; acepromazine; dothiepin; dextromoramide; fenoprofen; dextropropoxyphene; loxapine; betaxolol; propafenone; promethazine; thioproperazine; methadone; amoxapine; quinupramine; opipramol; cyproheptadine; brompheniramine; mefenidramine; protriptyline; flurbiprofen; tetrazepam; zorubicin; prazepam; alimemazine; loperamide; imipramine; desipramine; levomepromazine; hydroxyzine; niflumic acid; penbutolol; fluvoxamine; pimozide; daunorubicin; indomethacin; maprotiline; tropatenine; etodolac; fluoxetine; amitriptyline; nortriptyline; tioclomarol; diclofenac; mefloquine; trimipramine; chlorambucil; lidoflazine; ibuprofen; floctafenine; alpidem; loratadine; chlorpromazine; clomipramine; carpipramine; thioridazine; fentiazac; clemastine; mefenamic acid; fluphenazine; prochlorperazine; penfluridol; bepridil; terfenadine; trifluoperazine

REFERENCE

Tracqui,A.; Kintz,P.; Mangin,P. Systematic toxicological analysis using HPLC/DAD, *J.Forensic Sci.*, **1995**, *40*, 254–262.

Irbesartan

Molecular formula: $C_{25}H_{28}N_6O$
Molecular weight: 428.54
CAS Registry No.: 138402-11-6
Merck Index: 5097



SAMPLE

Matrix: blood, urine

Sample preparation: Condition a 3 mL 100 mg Isolute Cyano SPE cartridge (Jones Chromatography) with 2 mL MeOH and 2 mL 0.85% phosphoric acid. Plasma. Mix 1.0 mL 0.85% phosphoric acid and 100 µL 1 µg/mL IS in 0.85% phosphoric acid with 250 µL plasma. Pass the mixture slowly through the SPE cartridge, wash with 3 mL 0.85% phosphoric acid, wash with 1 mL hexane. Elute with 1 mL of mixture MeOH:0.85% phosphoric acid 50:50. Inject a 20 µL aliquot of the eluate. Urine. Mix 1.0 mL 0.85% phosphoric acid and 100 µL 1 µg/mL IS in 0.85% phosphoric acid with 250 µL urine. Pass the mixture slowly through the SPE cartridge, wash with 4 mL 0.85% phosphoric acid. Elute with 1 mL of mixture MeOH:0.85% phosphoric acid 50:50. Inject a 20 µL aliquot of the eluate.

HPLC VARIABLES

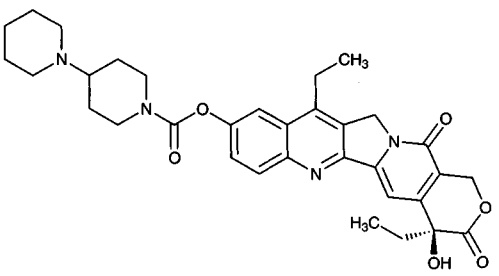
Column: 150 × 4.6 5 µm YMC-ODS-AQ
Mobile phase: MeCN:buffer 50:50 (Prepare buffer by adding 1 mL triethylamine to 1 L water, adjust pH to 3.5 with 85% phosphoric acid.)
Flow rate: 0.8
Injection volume: 20
Detector: F ex 250 em 371

CHROMATOGRAM
Retention time: 6.6
Internal standard: BMS-190462 (9.4)
Limit of quantitation: 1 ng/mL

KEY WORDS
plasma; urine; SPE; pharmacokinetics

REFERENCE
Chang,S.-Y.; Whigan,D.B.; Vachharajani,N.N.; Patel,R. High-performance liquid chromatographic assay for the quantitation of irbesartan (SR 47436/BMS-186295) in human plasma and urine, *J.Chromatogr.B*, **1997**, *702*, 149–155.

Irinotecan



Molecular formula: $C_{33}H_{38}N_4O_6$
Molecular weight: 586.69
CAS Registry No.: 97682-44-5, 100286-90-6 (HCl)
Merck Index: 5104

SAMPLE
Matrix: bile
Sample preparation: Dilute bile, inject an aliquot.

HPLC VARIABLES
Column: 300 × 7.2 TSKgel-ODS 80Tm (Tosoh)
Mobile phase: Gradient. MeOH:100 mM pH 4.0 phosphate buffer from 50:50 to 60:40 over 20 min, maintain at 60:40 for 20 min.
Flow rate: 3
Detector: UV 254

CHROMATOGRAM
Retention time: 16

OTHER SUBSTANCES
Extracted: metabolites

KEY WORDS
rat; preparative for metabolites

REFERENCE
Atsumi,R.; Suzuki,W.; Hakusui,H. Identification of the metabolites of irinotecan, a new derivative of camptothecin, in rat bile and its biliary excretion, *Xenobiotica*, **1991**, *21*, 1159–1169.

SAMPLE
Matrix: blood
Sample preparation: Mix 10 μL plasma with 100 μM diisopropyl fluorophosphate, 125 ng/mL camptothecin and 375μL MeOH with an automatic mixer (S-100, Taitec, Saitama, Japan) for 30 s, centrifuge at 3000 rpm for 10 min. Evaporate the supernatant on a Speed Vac Plus SC110A (Savant Instruments. Inc., Farmingdale, NY) and dissolve the residue in 200 μL THF: 50 mM pH 2.0 KH_2PO_4 containing 5 mM heptanesulfonate 25:75. Centrifuge at 10000 rpm for 3 min, inject 20 or 50 μL aliquot.

HPLC VARIABLES
Guard column: 15 × 3.5 5 μm TSK-GEL ODS-80TS (Toyo soda, Tokyo)
Column: 150 × 4.6 5 μm TSK-GEL ODS-80TS (Toyo soda, Tokyo)
Mobile phase: THF:50mM pH 4.0 KH_2PO_4 containing 5mM heptanesulfonate 25:75
Flow rate: 0.8
Injection volume: 20 or 50
Detector: F ex 370 em 430

CHROMATOGRAM
Internal standard: camptothecin

KEY WORDS
plasma; mouse; pharmacokinetics

REFERENCE
Ohdo,S.; Makinosumi,T.; Ishizaki,T.; Yukawa,E.; Higuchi,S.; Nakano,S.; Ogawa,N. Cell cycle-dependent chronotoxicity of irinotecan hydrochloride in mice, *J.Pharmacol.Exp.Ther.*, **1997**, *283*, 1383–1388.

SAMPLE
Matrix: blood
Sample preparation: Determination of lactone form. 1 mL Plasma + 100 μL 25 ng/mL IS in MeOH:10 mM HCl 40:60 + 800 mg solid NaCl, extract with 7.5 mL MeCN:n-butyl chloride 20:80 for 5 min, centrifuge at 4000 g for 2 min. Rotate quickly by hand to break the gels, centrifuge at 4000 g for 5 min. Mix the organic layer with 50 μL DMSO, dry under a gentle stream of nitrogen at 60° to approximately 50 μL. Reconstitute the residue in 100 μL MeOH and 100 μL perchloric acid:water 1:500, vortex for 5 s, inject a 100 μL aliquot. Total determination. Mix 250 μL plasma with 500 μL cold (-20°) MeOH:perchloric acid:water 20:1:20, centrifuge at 24000 g for 5 min, inject a 100 μL aliquot of the supernatant.

HPLC VARIABLES
Guard column: 4 × 4 5 μm LiChrospher 100 RP-18
Column: 100 × 4.6 5 μm Hypersil ODS
Mobile phase: MeOH:buffer 40:60 adjusted to pH 5.5 with HCl (A, lactone form determination) or MeOH:buffer 35:65 adjusted to pH 5.5 with HCl (B, total determination). (Buffer was 100 mM ammonium acetate containing 10 mM tetrabutylammonium sulfate.)
Column temperature: 50
Flow rate: 1
Injection volume: 100
Detector: F ex 355 em 515

CHROMATOGRAM
Retention time: 4.9 (A), 8.3 (B)
Internal standard: camptothecin (6.5, A)
Limit of quantitation: 200 pg/mL (lactone form), 2.0 ng/mL (total)

OTHER SUBSTANCES
Extracted: active metabolite
Noninterfering: acetaminophen, alizapride, codeine, dexamethasone, domperidone, metoclopramide, morphine, ranitidine

KEY WORDS
plasma; pharmacokinetics

REFERENCE
de Bruijn,P.; Verweij,J.; Loos,W.J.; Nooter,K.; Stoter,G.; Sparreboom,A. Determination of irinotecan (CPT-11) and its active metabolite SN-38 in human plasma by reversed-phase high-performance liquid chromatography with fluorescence detection, *J.Chromatogr.B*, **1997**, *698*, 277–285.

SAMPLE
Matrix: blood

Sample preparation: Add 1 mL MeCN:MeOH 50:50 to 500 μL plasma at -20°, vortex for 10 s. Centrifuge at 10000 g at 4° for 3 min, mix 70 μL supernatant with 70 μL mobile phase at 0°, vortex, inject a 20 μL aliquot.

HPLC VARIABLES
Guard column: 10 × 3 C18 (Chrompack, Netherlands)
Column: 150 × 4.6 3.5 μm Zorbax SB C18
Mobile phase: MeCN:100mM pH 6.4 ammonium acetate:triethylamine 15.6:80:0.1 containing 5 mM tetrabutylammonium phosphate
Flow rate: 1.5
Injection volume: 20
Detector: F ex 375 em 460

CHROMATOGRAM
Retention time: 5.7 (carboxylate form), 11.1 (lactone form)
Limit of quantitation: 1 ng/mL

OTHER SUBSTANCES
Extracted: metabolites (F ex 385 em 525)
Noninterfering: lurtotecan, topotecan

KEY WORDS
plasma; pharmacokinetics

REFERENCE
Herben,V.M.M.; Mazee,D.; van Gortel-van Zomeren,D.M.; Zeedijk,S.; Schellens,J.H.M.; ten Bokkel Huinink,W.W.; Beijnen,J.H. Sensitive determination of the carboxylate and lactone forms of the novel antitumor drug irinotecan and its active metabolite in plasma by HPLC, *J.Liq.Chromatogr.Rel.Technol.*, **1998**, *21*, 1541–1558.

SAMPLE
Matrix: blood
Sample preparation: Condition a 100 mg Bond Elut C18 SPE cartridge with 1 mL MeOH and 1 mL water at 0.5 mL/s. 100 μL Plasma + 50 μL 1 μg/mL camptothecin in 10 mM HCl + 850 μL 10 mM HCl, mix, add to the SPE cartridge at 2.4 mL/min, wash with 1.5 mL water at 6 mL/min, wash with 1 mL 10 mM HCl at 6 mL/min, elute with 1.5 mL acidic MeOH at 2.4 mL/min. Evaporate the eluate to dryness under a stream of nitrogen, reconstitute the residue in 250 μL mobile phase, inject an aliquot. (Prepare IS solution by sonicating 1 mg camptothecin in 25 mL MeOH at room temperature for 20 min, dilute to 10 μg/mL with water, dilute to 1 μg/mL with 10 mM HCl. Prepare acidic MeOH by mixing 100 μL concentrated HCl with 100 mL MeOH.)

HPLC VARIABLES
Guard column: 22 × 3.5 10 μm Nucleosil C18
Column: 300 × 3.9 10 μm Nucleosil octadecylsilane
Mobile phase: MeCN:100 mM KH_2PO_4 34:66 containing 3 mM sodium heptanesulfonate, adjusted to pH 4 with 1 M HCl
Flow rate: 1
Detector: F ex 380 em 500

CHROMATOGRAM
Retention time: 5.5
Internal standard: camptothecin (9)
Limit of detection: 1 ng/mL

OTHER SUBSTANCES
Extracted: metabolites

KEY WORDS
plasma; pharmacokinetics; SPE

REFERENCE

Barilero,I.; Gandia,D.; Armand,J.-P.; Mathieu-Boué,A.; Ré,M.; Chabot,G.G. Simultaneous determination of the camptothecin analogue CPT-11 and its active metabolite SN-38 by high-performance liquid chromatography: application to plasma pharmacokinetic studies in cancer patients, *J.Chromatogr.*, **1992**, *575*, 275–280.

SAMPLE

Matrix: blood

Sample preparation: Evaporate 50 μL 1 μg/mL camptothecin in acetone into the bottom of a tube under a stream of nitrogen, add 50 μL plasma, add 100 μL ice-cold MeCN:MeOH 50:50, vortex for 5 s, centrifuge at 8000 g briefly. Remove a 100 μL aliquot of the supernatant and add it to 70 μL 75 mM pH 6.4 ammonium acetate buffer, vortex briefly, inject a 5-20 μL aliquot.

HPLC VARIABLES

Guard column: Guard-Pak Nova-Pak C18

Column: 100 × 5 4 μm Nova-Pak Radial-Pak C18

Mobile phase: MeCN:75 mM pH 6.4 ammonium acetate buffer 22:78 containing 5 mM tetrabutylammonium phosphate (PIC A)

Flow rate: 1.5

Injection volume: 5-20

Detector: F ex 355 em 515

CHROMATOGRAM

Retention time: 4.2 (carboxylate form), 8.2 (lactone form)

Internal standard: camptothecin (6.5 (carboxylate form), 10.5 (lactone form))

Limit of quantitation: 10 ng/mL

OTHER SUBSTANCES

Extracted: metabolites

KEY WORDS

plasma; pharmacokinetics; carboxylate form is the inactive form

REFERENCE

Rivory,L.P.; Robert,J. Reversed-phase high-performance liquid chromatographic method for the simultaneous quantitation of the carboxylate and lactone forms of the camptothecin derivative irinotecan, CPT-11 and its metabolite SN-38 in plasma, *J.Chromatogr.B*, **1994**, *661*, 133–141.

SAMPLE

Matrix: blood

Sample preparation: 200 μL Plasma + 50 μL 1.25 μg/mL camptothecin in MeOH:0.5 M HCl 97.5:2.5 + 750 μL MeOH, vortex for 10 s, centrifuge at 20° at 10000 g for 5 min. Remove the supernatant and evaporate it to dryness under reduced pressure, reconstitute with 400 μL mobile phase adjusted to pH 2.0, vortex for 10 s, centrifuge at 20° at 10000 g for 5 min, inject a 100 μL aliquot of the supernatant.

HPLC VARIABLES

Guard column: 15 × 3.2 TSK guardgel ODS-120T

Column: 150 × 4.6 5 μm TSK gel ODS-80Ts

Mobile phase: MeCN:50 mM Na_2HPO_4 28:72 containing 5 mM sodium 1-heptanesulfonate, adjusted to pH 3.0 with orthophosphoric acid (Prepared by dissolving 17.9 g $Na_2HPO_4.12H_2O$ and 1.1 g sodium 1-heptanesulfonate hydrate in 1 L water and adding 388 mL MeCN, adjust pH to 3.0 with orthophosphoric acid.)

Column temperature: 30

Flow rate: 1

Injection volume: 100

Detector: F ex 380 em 556 or ex 370 em 430

CHROMATOGRAM

Retention time: 5.4

Internal standard: camptothecin (8.8)

Limit of quantitation: 30 ng/mL

OTHER SUBSTANCES
Extracted: metabolites

KEY WORDS
plasma; pharmacokinetics

REFERENCE
Sumiyoshi,H.; Fujiwara,Y.; Ohune,T.; Yamaoka,N.; Tamura,K.; Yamakido,M. High-performance liquid chromatographic determination of irinotecan (CPT-11) and its active metabolite (SN-38) in human plasma, *J.Chromatogr.B*, **1995**, *670*, 309–316.

SAMPLE
Matrix: blood, feces, urine
Sample preparation: Plasma, urine. Thaw frozen plasma or urine sample in a waterbath, vortex, add 250 μL plasma or 250 μL plasma:diluted urine 50:50 to 500 μL MeOH:5% (w/v) aqueous perchloric acid 50:50. Mix for 5 min, centrifuge at 24000 g for 5 min, dilute the upper aqueous layer from plasma and urine extracts 2-fold and 10-fold, respectively, with mobile phase, inject a 100-200 μL aliquot. Feces. Homogenize feces with 5 volumes of 5% (w/v) perchloric acid in water using five 1 min bursts from an Ystral X1020 tissue homogenizer at 20500 r.p.m. Centrifuge at 24000 g for 10 min, dilute with one volume of drug-free human plasma, and process further as described for the urine sample.

HPLC VARIABLES
Guard column: 4 × 4 LiChroCart endcapped RP-18 Merck
Column: 100 × 4.6 5 μm Hypersil ODS
Mobile phase: MeOH:100 mM ammonium acetate containing 10 mM tetrabutylammonium sulphate 30:70, pH adjusted to 5.3 with HCl
Column temperature: 50
Flow rate: 1.0
Injection volume: 100-200
Detector: F ex 355 em 515

CHROMATOGRAM
Retention time: 16.1
Limit of quantitation: 10 ng/mL (plasma), 100 ng/mL (feces, urine)

OTHER SUBSTANCES
Extracted: metabolites

KEY WORDS
pharmacokinetics; plasma

REFERENCE
Sparreboom,A.; de Bruijn,P.; de Jonge,M.J.A.; Loos,W.J.; Stoter,G.; Verweij,J.; Nooter,K. Liquid chromatographic determination of irinotecan and three major metabolites in human plasma, urine and feces, *J.Chromatogr.B*, **1998**, *712*, 225–235.

SAMPLE
Matrix: blood, tissue
Sample preparation: Condition an Analytichem C18 SPE cartridge with 1.5 mL MeOH and 1.5 mL water. Homogenize (PTFE homogenizer) tissue with four volumes ice-cold 150 mM KCl, centrifuge at 2° at 9000 g for 20 min. Dilute plasma, serum, or tissue homogenate supernatant 10-fold with 100 mM HCl, add to the SPE cartridge, elute the contents of the SPE cartridge on to the column with the mobile phase.

HPLC VARIABLES
Guard column: RP-18
Column: 250 × 4 LiChrosorb RP-18
Mobile phase: MeCN:EtOH:0.8% ammonium carbonate 50:25:25
Column temperature: 50
Flow rate: 1

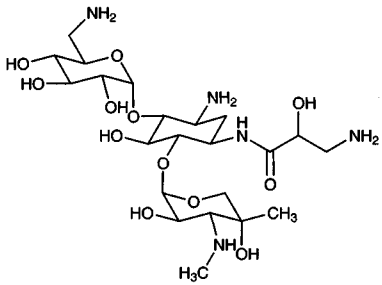
Detector: F ex 373 em 428

CHROMATOGRAM
Retention time: 6.6
Limit of detection: 1 ng

KEY WORDS
mouse; plasma; serum; liver; epithelium; pharmacokinetics; SPE

REFERENCE
Kaneda,N.; Nagata,H.; Furuta,T.; Yokokura,T. Metabolism and pharmacokinetics of the camptothecin analogue CPT-11 in the mouse, *Cancer Res.*, **1990**, *50*, 1715–1720.

Isepamicin



Molecular formula: $C_{22}H_{43}N_5O_{12}$
Molecular weight: 569.61
CAS Registry No.: 58152-03-7, 67814-76-0 (sulfate)
Merck Index: 5121

SAMPLE
Matrix: blood
Sample preparation: Mix 1 mL plasma with 0.5-5.0 μg IS and 2 mL EtOH. Add 7 mL dichloromethane and 1 mL water, mix, centrifuge. Inject an aliquot of the aqueous supernatant onto column A, elute to waste with mobile phase A, after 4 min elute the contents of column A onto column B with mobile phase B, elute column B with mobile phase B. Mix the effluent from column B with o-phthalaldehyde at 0.2 mL/min and monitor.

HPLC VARIABLES
Column: A 3.9 × 4.0 10 μm Guard Pak Cyano (Waters); B 150 × 4.6 5 μm Shandon Hypersil C18
Mobile phase: A 17 mM acetic acid containing 10 mM hexanesulfonic acid; B 17 mM acetic acid containing 10 mM hexanesulfonic acid, 100 mM sodium acetate, and 3.53 M MeOH
Detector: F ex 338 em 450 (cut-off filter) following post-column reaction. The column effluent mixed with o-phthalaldehyde pumped at 0.2 mL/min and the mixture flowed to the detector.

CHROMATOGRAM
Retention time: 7.4
Internal standard: dibekacin (9.5)
Limit of quantitation: 100 ng/mL

OTHER SUBSTANCES
Simultaneous: aspirin, caffeine, chlorpheniramine, gentamicin, neomycin, netilmicin, sisomicin

KEY WORDS
plasma; pharmacokinetics; comparison with RIA and microbiological assay; post-column reaction; column-switching

REFERENCE
Lin,C.-.; Veals,J.; Korduba,C.; Hilbert,M.J.; Nomeir,A. Analysis of isepamicin in human plasma by radioimmunoassay, microbiologic assay, and high-performance liquid chromatography, *Ther.Drug Monit.*, **1997**, *19*, 675–681.

SAMPLE
Matrix: blood

Sample preparation: Dry blood on gauze, soak gauze in 500 μL 500 mM Na_2HPO_4 at 35° for 30 min, inject a 50 μL aliquot.

HPLC VARIABLES
Column: 300 × 3.9 5 μm μBondapak C18
Mobile phase: 16 mM Sodium sulfate containing 5 mM 1-heptanesulfonic acid (PIC B-7)
Flow rate: 0.6
Injection volume: 50
Detector: F ex 360 em 440 following post-column reaction. The column effluent was mixed with reagent pumped at 0.3 mL/min and flowed through a 5 m × 0.25 mm i.d. coil of PTFE tubing to the detector. (Prepare reagent by dissolving 300 mg o-phthalaldehyde in 500 mL MeOH, add 1.25 mL β-mercaptoethanol, add 500 mL 400 mM pH 10.4 potassium borate buffer.)

CHROMATOGRAM
Retention time: 12
Limit of detection: 100 ng/mL

KEY WORDS
post-column reaction; dried blood

REFERENCE
Shoshihara,M.; Kase,K.; Yoshizawa,E.; Takao,M.; Fujimoto,T. Column liquid chromatographic determination of isepamicin in nasal cavity using gauze, *J.Chromatogr.*, **1990**, *529*, 473–478.

SAMPLE
Matrix: blood
Sample preparation: 50 μL Plasma + 20 μL 10 mg/mL gentamicin C1a in water + 50 μL buffer, vortex for 15 s, add 200 μL MeCN, vortex for 20 s, centrifuge at 2000 g for 5 min. Filter (Millex-HV4) the supernatant. Heat 200 μL filtrate and 20 μL 250 mg/mL 1-fluoro-2,4-dinitrobenzene in MeCN at 80° for 1 h, cool, inject a 50 μL aliquot. (Buffer was 3.81 g disodium tetraborate decahydrate in water, adjust pH to 10 with NaOH, make up to 100 mL with water.)

HPLC VARIABLES
Guard column: 25 × 4 10 μm LiChroCART RP 18
Column: 250 × 4 5 μm LiChrosorb RP 18
Mobile phase: MeCN:water 70:30 containing 1 mL/L acetic acid
Flow rate: 2
Injection volume: 50
Detector: UV 365

CHROMATOGRAM
Retention time: 13.0
Internal standard: gentamicin C1a (10.0)
Limit of quantitation: 500 ng/mL

OTHER SUBSTANCES
Noninterfering: ampicillin, aspirin, captopril, cefazolin, cefotaxime, ceftazidime, ceftriaxone, cephalosporins, chlorpromazine, diazepam, heparin, propranolol, sulfamethoxazole, sulpiride, trimethoprim, verapamil

KEY WORDS
plasma; guinea pig; human; derivatization

REFERENCE
Dionisotti,S.; Bamonte,F.; Scaglione,F.; Ongini,E. Simple measurement of isepamicin, a new aminoglycoside antibiotic, in guinea pig and human plasma, using high-performance liquid chromatography with ultraviolet detection, *Ther.Drug Monit.*, **1991**, *13*, 73–78.

SAMPLE
Matrix: blood, dialysate, urine

Sample preparation: Plasma. Condition a 3 mL Baker cyanopropylsilane CN SPE cartridge with 2 mL MeOH, 2 mL water, and 2 mL buffer. 1 mL Plasma + 100 μL 100 μg/mL dibekacin in water, vortex for 15 s, add 1 mL buffer, vortex for 15 s, centrifuge at 3100 g at 4° for 7 min, add to SPE cartridge, wash with 500 μL water, wash with 250 μL mobile phase, elute to dryness. Elute with 250 μL mobile phase, inject an aliquot of the eluate. Urine, dialysate. Dilute 1:100 with water, add 100 μL 100 μg/mL dibekacin per 1 mL of sample, mix well, inject a 100 μL aliquot. (Buffer was 0.94 g sodium hexanesulfonate in 300 mL water, add 500 μL glacial acetic acid, dilute to 500 mL with water.)

HPLC VARIABLES

Guard column: 10 × 4.6 5 μm Hypersil C18
Column: 150 × 4.6 5 μm Hypersil C18
Mobile phase: MeOH:buffer 10:90 (Buffer was 3.76 g sodium hexanesulfonate + 28.4 g sodium sulfate in 2 L water, acidify to pH 3.4 with 2 mL glacial acetic acid.)
Column temperature: 25
Flow rate: 1.1
Injection volume: 100
Detector: F ex 338 em 418 (bandpass filter) following post-column reaction. The column effluent mixed with the reagent pumped at 0.4 mL/min and the mixture flowed through a 3 m × 0.05 mm i.d. knitted PTFE reaction coil at 25° to the detector (Derivatizing reagent was 0.4 g o-phthalaldehyde in 3 mL MeOH added to 390 mL buffer, add 2 mL β-mercaptoethanol, make up to 500 mL with water, store at 4°. Buffer was 1 M pH 10.4 borate from equal volumes of 1 M KOH and boric acid.)

CHROMATOGRAM

Retention time: 6.7
Internal standard: dibekacin (17)
Limit of quantitation: 100 ng/mL

OTHER SUBSTANCES

Simultaneous: kanamycin, gentamicin, tobramycin, netilmicin

KEY WORDS

post-column reaction; SPE; plasma

REFERENCE

Maloney,J.A.; Awni,W.M. High-performance liquid chromatographic determination of isepamicin in plasma, urine and dialysate, *J.Chromatogr.*, **1990**, *526*, 487–496.

SAMPLE

Matrix: blood, urine
Sample preparation: 1 mL Plasma or urine + 0.5-5 μg dibekacin + 2 mL EtOH, mix, add 7 mL dichloromethane, add 1 mL water, centrifuge, inject an aliquot of the aqueous supernatant on to column A and elute to waste with mobile phase A, after 4 min elute the contents of column A on to column B with mobile phase B, monitor the effluent from column B.

HPLC VARIABLES

Column: A 3.9 × 4 10 μm Guard Pak with Cyano insert; B 150 × 4.6 5 μm Hypersil C18
Mobile phase: A 17 mM Acetic acid containing 10 mM hexanesulfonate; B MeOH:buffer 14.3: 85.7 (Mobile phase contained 100 mM sodium acetate, 17 mM acetic acid, 10 mM hexanesulfonate, and 3.53 M MeOH.)
Detector: F ex 338 (band-pass filter) em 418-700 and 450 cut-off filters following post-column reaction with o-phthalaldehyde derivatizing reagent pumped at 0.2 mL/min.

CHROMATOGRAM

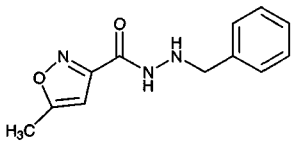
Internal standard: dibekacin
Limit of quantitation: 100 ng/mL

KEY WORDS

plasma; pharmacokinetics; post-column reaction; column-switching

REFERENCE
Lin,C.-C.; Radwanski,E.; Korduba,C.; Cayen,M.; Affrime,M. Pharmacokinetics of intravenously administered isepamicin in men, *Antimicrob.Agents Chemother.*, **1995**, *39*, 2774–2778.

Isocarboxazid



Molecular formula: $C_{12}H_{13}N_3O_2$
Molecular weight: 231.25
CAS Registry No.: 59-63-2
Merck Index: 5172
Lednicer No.: 1 233

SAMPLE
Matrix: blood
Sample preparation: 1 mL Plasma + 200 µL 1.5 M NaOH + 10 µL 200 µg/mL IS in 100 mM HCl, vortex, add 5 mL hexane:ethyl acetate 80:20, shake mechanically at low speed for 10 min, centrifuge at 10° at 1100 g for 10 min. Remove the organic layer and add it to 500 µL 2 M HCl, shake mechanically for 5 min, centrifuge at 10° at 1100 g for 5 min. Remove the aqueous phase and add it to 200 µL saturated K_2HPO_4, vortex, inject a 50 µL aliquot.

HPLC VARIABLES
Column: 150 × 4.6 5 µm LC-18 (Supelco)
Mobile phase: MeOH:5 mM octanesulfonic acid 50:50
Flow rate: 1
Injection volume: 50
Detector: UV 230

CHROMATOGRAM
Retention time: 6.4
Internal standard: 5-methyl-3-isoxazolecarboxylic acid 2-(2-propyl-1-phenyl)hydrazide (Ro 5-1226) (15.5)
Limit of quantitation: 100 ng/mL

KEY WORDS
plasma; dog; pharmacokinetics

REFERENCE
Powell,M.L.; Town,C.; Henderson,L.; Buck,C. Determination of marplan in human plasma using high-performance liquid chromatography, *J.Chromatogr.*, **1990**, *529*, 237–244.

SAMPLE
Matrix: solutions

HPLC VARIABLES
Column: 250 × 4.6 Zorbax RX
Mobile phase: Gradient. A was 10 mL concentrated orthophosphoric acid and 7 mL triethylamine in 1 L water. B was 10 mL concentrated orthophosphoric acid and 7 mL triethylamine in 200 mL water, make up to 1 L with MeCN. A:B from 100:0 to 0:100 over 30 min, maintain at 0:100 for 5 min.
Column temperature: 30
Flow rate: 2
Detector: UV 210

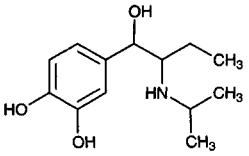
OTHER SUBSTANCES

Also analyzed: acepromazine, acetaminophen, acetophenazine, albuterol, aminophylline, amitriptyline, amobarbital, amoxapine, amphetamine, amylocaine, antipyrine, aprobarbital, aspirin, atenolol, atropine, avermectin, barbital, benzocaine, benzoic acid, benzotropine, benzphetamine, berberine, bibucaine, bromazepan, brompheniramine, buprenorphine, buspirone, butabarbital, butacaine, butethal, caffeine, carbamazepine, carbromal, chloramphenicol, chlordiazepoxide, chloroquine, chlorothiazide, chloroxylenol, chlorphenesin, chlorpheniramine, chlorpromazine, chlorpropamide, chlortetracycline, cimetidine, cinchonidine, cinchonine, clenbuterol, clonazepam, clonixin, clorazepate, cocaine, codeine, colchicine, cortisone, coumarin, cyclazocine, cyclobenzaprine, cyclothiazide, cyheptamide, cymarin, danazol, danthron, dapsone, debrisoquine, desipramine, dexamethasone, dextromethorphan, dextropropoxyphene, diamorphine, diazepam, diclofenac, diethylpropion, diethylstilbestrol, diflunisal, digitoxin, digoxin, diltiazem, diphenhydramine, diphenoxylate, diprenorphine, dipyrone, disulfiram, dopamine, doxapram, doxepin, dronabinol, ephedrine, epinephrine, epinine, estradiol, estriol, estrone, ethacrynic acid, ethosuximide, etonitazene, etorphine, eugenol, famotidine, fenbendazole, fencamfamine, fenoprofen, fenproporex, fentanyl, flubendazole, flufenamic acid, flunitrazepam, 5-fluorouracil, fluoxymesterone, fluphenazine, furosemide, gentisic acid, gitoxigenin, glipizide, glunixin, glutethimide, glybenclamide, guaiacol, halazepam, haloperidol, hydrochlorothiazide, hydrocodone, hydrocortisone, hydromorphone, hydroxyquinoline, ibogaine, ibuprofen, iminostilbene, imipramine, indomethacin, isoniazid, isoproterenol, isoxsuprine, ivermectin, ketamine, ketoprofen, kynurenic acid, levorphanol, lidocaine, lorazepam, lormetazepam, loxapine, mazindol, mebendazole, meclizine, meclofenamic acid, medazepam, mefenamic acid, megestrol, mepacrine, meperidine, mephentermine, mephenytoin, mephesin, mephobarbital, mepivacaine, mescaline, mesoridazine, methadone, methamphetamine, methapyrilene, methaqualone, methazolamide, methocarbamol, methoxamine, methsuximide, methyl salicylate, methyldopa, methyldopamine, methylphenidate, methylprednisolone, methyltestosterone, methyprylon, metoprolol, mibolerone, morphine, nadolol, nalorphine, naloxone, naltrexone, naphazoline, naproxen, nefopam, niacinamide, nicotine, niacin, nifedipine, niflumic acid, nitrazepam, norepinephrine, nortriptyline, noscapine, nylidrin, oxazepam, oxycodone, oxymorphone, oxyphenbutazone, oxytetracycline, papaverine, pargyline, pemoline, pentazocine, pentobarbital, persantine, phenacetin, phenazocine, phenazopyridine, phencyclidine, phendimetrazine, phenelzine, pheniramine, phenobarbital, phenothiazine, phensuximide, phentermine, phenylbutazone, phenylephrine, phenylpropanolamine, piperocaine, prazepam, prednisolone, primidone, probenecid, progesterone, propiomazine, propranolol, propylparaben, pseudoephedrine, puromycin, pyrilamine, pyrithyldione, quazepam, quinaldic acid, quinidine, quinine, ranitidine, recinnamine, reserpine, resorcinol, saccharin, albuterol, salicylamide, salicylic acid, scopolamine, scopoletin, secobarbital, strychnine, sulfacetamide, sufadiazine, sulfadimethoxine, sulfaethidole, sulfamerazine, sulfamethazine, sulfamethoxizole, sulfanilamide, sulfapyridine, sulfasoxizole, sulindac, tamoxifen, temazepam, testosterone, tetracaine, tetracycline, tetramisole, thebaine, theobromine, theophylline, thiabendazole, thiamine, thiamylal, thiobarbituric acid, thioridazine, thiosalicylic acid, thiothixene, thymol, tolazamide, tolazoline, tobutamide, tolmetin, tranylcypromine, triamcinolone, tribenzylamine, trichloromethiazide, trifluoperazine, trihexyphenidyl, trimethoprim, tripelennamine, triprolidine, tropacocaine, tyramine, verapamil, vincamine, warfarin, yohimbine, zoxazolamine

REFERENCE

Hill,D.W.; Kind,A.J. Reversed-phase solvent gradient HPLC retention indexes of drugs, *J.Anal.Toxicol.*, **1994**, *18*, 233–242.

Isoetharine



Molecular formula: $C_{13}H_{21}NO_3$
Molecular weight: 239.31
CAS Registry No.: 530-08-5, 2576-92-3 (HCl), 7279-75-6 (mesylate)
Merck Index: 5185
Lednicer No.: 2 9

SAMPLE

Matrix: blood
Sample preparation: 25-150 μL Plasma + 100 μL 50 ng/mL colterol mesylate in 25 mM sulfuric acid + 2 mL 2% boric acid + 2 g ammonium sulfate + 10 mL 0.5% di(2-ethylhexyl)phosphoric acid in benzene (Caution! Benzene is a carcinogen!), shake, centrifuge. Remove the organic phase and add it to 130 μL 25 mM sulfuric acid, shake, centrifuge, inject a 100 μL aliquot of the aqueous phase.

HPLC VARIABLES

Column: 250 × 4.6 5 μm Ultrasphere ODS
Mobile phase: MeOH:buffer 12.5:87.5 (Buffer was 100 mM sodium sulfate adjusted to pH 2.8 phosphoric acid then adjusted to pH 3.0 with NaOH.)
Flow rate: 1.2
Injection volume: 100
Detector: E, Bioanalytical Systems LC-4, TL-5 glassy carbon electrode +0.60 V, Ag/AgCl reference electrode

CHROMATOGRAM

Retention time: 9.3
Internal standard: colterol mesylate (7.5)
Limit of quantitation: 0.5 ng/mL

KEY WORDS

plasma; rat; pharmacokinetics

REFERENCE

Park,G.B.; Koss,R.F.; Utter,J.; Mayes,B.A.; Edelson,J. Determination of isoetharine in plasma by reversed-phase chromatography with amperometric detection, *J.Pharm.Sci.*, **1982**, *71*, 932–934.

SAMPLE

Matrix: solutions

HPLC VARIABLES

Column: 250 × 4.6 Chirex 3020 (Phenomenex)
Mobile phase: Hexane:1,2-dichloroethane:EtOH/trifluoroacetic acid 55:35:10 (EtOH/trifluoroacetic acid was premixed 20:1.)
Flow rate: 0.7-1
Injection volume: 20
Detector: UV 282

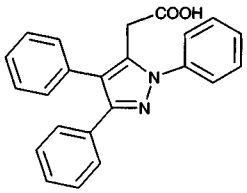
KEY WORDS

chiral; α = 1.21 for enantiomers

REFERENCE

Cleveland,T. Pirkle-concept chiral stationary phases for the HPLC separation of pharmaceutical racemates, *J.Liq.Chromatogr.*, **1995**, *18*, 649–671.

Isofezolac



Molecular formula: $C_{23}H_{18}N_2O_2$
Molecular weight: 354.41
CAS Registry No.: 50270-33-2
Merck Index: 5188

SAMPLE

Matrix: blood, urine
Sample preparation: Plasma. 1 mL Plasma + 1 mL 100 mM pH 4.4 citrate buffer + 100 μL 40 μg/mL IS in MeOH + 15 mL diethyl ether, agitate, centrifuge at 3000 g for 5 min. Remove the organic layer and evaporate it to dryness under a stream of nitrogen at 40°, reconstitute the residue in 0.5-3 mL mobile phase, inject a 20 μL aliquot. Urine. 100 μL Urine + 1 mL 100 mM pH 4.4 citrate buffer + 100 μL 40 μg/mL IS in MeOH + 100 μL enzyme solution containing 100000 U/mL β-glucuronidase and 1000000 U/mL arylsulfatase (I.B.F.), heat at 37° for 16 h, add 15 mL diethyl ether, agitate, centrifuge at 3000 g for 5 min. Remove the organic layer and evaporate it to dryness under a stream of nitrogen at 40°, reconstitute the residue in 1-2 mL mobile phase, inject a 20 μL aliquot.

HPLC VARIABLES

Column: 150 × 4.6 5 μm LiChrosorb RP 8
Mobile phase: MeCN:water:200 mM pH 3 phosphate buffer 65:15:20
Flow rate: 1.5
Injection volume: 20
Detector: F ex 273 em 335 or UV 265

CHROMATOGRAM

Retention time: 2.7
Internal standard: 1-phenyl-3,4-di-p-chlorophenylpyrazole-5-acetic acid (4.5)
Limit of detection: 10 ng/mL (F)

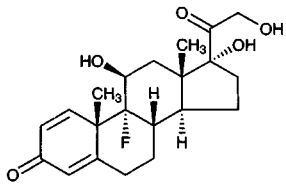
KEY WORDS

plasma; pharmacokinetics

REFERENCE

Bannier,A.; Brazier,J.L. Determination of isofezolac in biological fluids by reversed-phase liquid column chromatography, *J.Chromatogr.*, **1980**, *182*, 369–377.

Isoflupredone



Molecular formula: $C_{21}H_{27}FO_5$
Molecular weight: 378.44
CAS Registry No.: 338-95-4, 338-98-7 (acetate)
Merck Index: 5190

SAMPLE

Matrix: bulk, formulations
Sample preparation: Cream. Weigh out cream containing 1 mg diflorasone diacetate, add 30 mL 40 μg/mL isoflupredone acetate in water-saturated chloroform, shake for 30 min, centrifuge at 2000 rpm for 15 min, inject a 10 μL aliquot of the lower chloroform layer. Ointment. Weigh out ointment containing 0.5 mg diflorasone diacetate, add 15 mL 40 μg/mL isoflupredone acetate in water-saturated chloroform, shake for 30 min, centrifuge at 2000 rpm for 15 min, inject a 10 μL aliquot of the lower chloroform layer. Bulk. Dissolve 1.5 mg bulk drug in 50 mL 40 μg/mL isoflupredone acetate in water-saturated chloroform, inject a 10 μL aliquot.

HPLC VARIABLES
Column: 100 × 4.6 3 μm silica gel (Perkin-Elmer part 0258-1500)
Mobile phase: Butyl chloride:dichloromethane:THF:acetic acid 70:25:2:3 (Butyl chloride and dichloromethane were saturated with water.)
Flow rate: 2.5
Injection volume: 10
Detector: UV 254

CHROMATOGRAM
Retention time: 24 (isoflupredone acetate)
Internal standard: isoflupredone acetate

OTHER SUBSTANCES
Simultaneous: related compounds, diflorasone diacetate

KEY WORDS
cream; ointment; normal phase; isoflupredone acetate is IS

REFERENCE
Shaw,M.C.; Vanderwielen,A.J. Liquid chromatographic assay for diflorasone diacetate in cream and ointment formulations, *J.Pharm.Sci.*, **1984**, *73*, 1606–1608.

SAMPLE
Matrix: solutions

HPLC VARIABLES
Column: 250 × 4.6 5 μm SI-100 (Brownlee)
Mobile phase: Butyl chloride:THF:MeOH:glacial acetic acid 88:2.5:2.5:2.5 (Butyl chloride was 50% water saturated.)
Injection volume: 20
Detector: UV 254

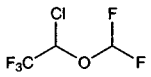
CHROMATOGRAM
Retention time: 16 (isoflupredone), 10 (isoflupredone acetate

OTHER SUBSTANCES
Simultaneous: bromoprednisolone acetate, prednisolone, fluoroprednisone acetate

KEY WORDS
normal phase

REFERENCE
Kane,M.P.; Tsuji,K. Radiolytic degradation scheme for ^{60}Co-irradiated corticosteroids, *J.Pharm.Sci.*, **1983**, *72*, 30–35.

Isoflurane



Molecular formula: $C_3H_2ClF_5O$
Molecular weight: 184.49
CAS Registry No.: 26675-46-7
Merck Index: 5191

SAMPLE
Matrix: solutions
Sample preparation: Mix 50 μL phosphate buffer containing isoflurane and 50 μL 0.05 mM toluene in MeOH, inject a 20 μL aliquot.

HPLC VARIABLES
Guard column: RCSS Guard-Pak μBondapak C18 precolumn cartridge
Column: 100 × 8 4 μm Nova-Pak C18 Radial Compression Module
Mobile phase: MeOH:water 50:50
Flow rate: 3.5
Injection volume: 20
Detector: UV 203

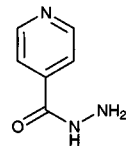
CHROMATOGRAM
Retention time: 5
Internal standard: toluene (12)
Limit of detection: 0.2 mM

OTHER SUBSTANCES
Simultaneous: enflurane, halothane

KEY WORDS
buffer

REFERENCE
Janicki,P.K.; Erskine,W.A.R.; James,M.F.M. High-performance liquid chromatographic method for the direct determination of the volatile anaesthetics halothane, isoflurane and enflurane in water and in physiological buffer solutions, *J.Chromatogr.*, **1990**, *518*, 250–253.

Isoniazid



Molecular formula: $C_6H_7N_3O$
Molecular weight: 137.14
CAS Registry No.: 54-85-3
Merck Index: 5203
Lednicer No.: 1 254

SAMPLE
Matrix: blood
Sample preparation: 2 mL Plasma + 100 μL 30μg/mL IS in water, mix, add 2 g ammonium sulfate, add 40 mL water saturated n-butanol:chloroform 30:70, shake for 10 min, centrifuge at 500 g for 10 min. Transfer the organic layer into a tube, add 1 mL 1 M sulfuric acid, shake for 10 min, centrifuge at 500 g for 10 min, inject a 250 μL aliquot of the upper aqueous layer. (Caution! Chloroform is a carcinogen !)

HPLC VARIABLES
Guard column: 10μm μBondapak C18
Column: 115 × 8 5 μm μBondapak C18 radial compression
Mobile phase: EtOH:1 mM dioctyl sulfosuccinate 45:55, adjusted to pH 2.50
Flow rate: 4
Injection volume: 250
Detector: UV 254

CHROMATOGRAM
Retention time: 10.7
Internal standard: 1-benzoyl-2-isonicotinoylhydrazine (8.9)
Limit of detection: 100 ng/mL

OTHER SUBSTANCES
Extracted: acetylisoniazid

KEY WORDS
plasma

REFERENCE
Holdiness,M.R. High pressure liquid chromatographic determination of isoniazid and acetylisoniazid in human plasma, *J.Liq.Chromatogr.*, **1982**, *5*, 707–714.

SAMPLE
Matrix: blood
Sample preparation: 2 mL Plasma + 50 μL 50 μg/mL phenelzine + 400 μL 10% acetic acid + 7 mL diethyl ether:dichloromethane 2:1, shake, centrifuge at 2059 g for 10 min. Remove the aqueous layer and add it to 600 μL 10% acetic acid, add 300 μL 0.1% salicaldehyde in EtOH, heat at 60° for 30 min, cool to room temperature, add 1 mL 1 M K_2PO_4 (sic), extract with 7 mL diethyl ether, centrifuge at 2059 g for 10 min, repeat extraction. Combine the organic layers and evaporate them to dryness under a stream of nitrogen at 40°, reconstitute the residue in 200 μL buffer, inject a 20 μL aliquot. (Buffer was 5 mM heptanesulfonic acid in MeCN:water: triethylamine 70:30:0.4.)

HPLC VARIABLES
Guard column: 50 × 4.6 30 μm C8
Column: 250 × 4.6 Spherisorb S5 ODS2 C18
Mobile phase: Gradient. MeCN:buffer:water 0:75:25 for 5 min, 15:85:0 for 12 min (step gradient). (Buffer was 5 mM heptanesulfonic acid in MeCN:water:triethylamine 70:30:0.4.)
Flow rate: 1
Injection volume: 20
Detector: UV 280

CHROMATOGRAM
Retention time: 4.68
Internal standard: phenelzine (11.09)
Limit of detection: 250 ng/mL

OTHER SUBSTANCES
Extracted: hydrazine, monoacetylhydrazine

KEY WORDS
derivatization

REFERENCE
Walubo,A.; Smith,P.; Folb,P.I. Comprehensive assay for pyrazinamide, rifampicin and isoniazid with its hydrazine metabolites in human plasma by column liquid chromatography, *J.Chromatogr.B*, **1994**, *658*, 391–396.

SAMPLE
Matrix: blood
Sample preparation: 250 μL Plasma + 150 μL 10% zinc sulfate in water, mix, centrifuge at 1000 g for 1 min. Remove a 250 μL aliquot of the supernatant and add it to 100 μL MeOH, mix, centrifuge, inject a 5 μL aliquot of the supernatant.

HPLC VARIABLES
Column: 250 × 4.6 5 μm LC-CN (Supelco)
Mobile phase: Isopropanol:water 5:95 containing 5 g/L ammonium formate
Flow rate: 1
Injection volume: 5
Detector: E, ESA Model 5100A, Model 5011 analytical cell with first detector +0.6 V and second (monitored) detector +0.8 V, Model 5020 guard cell +1.0 V between pump and injector

CHROMATOGRAM
Retention time: 4.4
Limit of detection: 100 ng/mL

KEY WORDS
rat; plasma

REFERENCE
Hansen,E.B.,Jr.; Dooley,K.L.; Thompson,H.C.,Jr. High-performance liquid chromatographic analysis of the antituberculosis drugs aconiazide and isoniazid, *J.Chromatogr.B*, **1995**, *670*, 259–266.

SAMPLE
Matrix: blood
Sample preparation: 250 μL Plasma + 50 μL 1.5% p-hydroxybenzaldehyde in MeOH + 40 μL 20% trichloroacetic acid in water, vortex thoroughly, centrifuge at 8000 g for 10 min, let stand on ice for 30 min, centrifuge at 8000 g for 10 min, inject a 30 μL aliquot of the supernatant.

HPLC VARIABLES
Guard column: 20 × 2 30-40 μm Perisorb RP18
Column: 100 × 8 4 μm Radial-Pak Novapak C18
Mobile phase: MeOH:water:20% tetraethylammonium hydroxide:70% perchloric acid 24:76:0.05:0.05, apparent pH 2.3
Flow rate: 2
Injection volume: 30
Detector: UV 350

CHROMATOGRAM
Retention time: 4.4
Limit of detection: <500 ng/mL

KEY WORDS
plasma; pharmacokinetics

REFERENCE
Schall,R.; Müller,F.O.; Duursema,L.; Groenewoud,G.; Hundt,H.K.L.; Middle,M.V.; Mogilnicka,E.M.; Swart,K.J. Relative bioavailability of rifampicin, isoniazid and ethambutol from a combination tablet vs. concomitant administration of a capsule containing rifampicin and a tablet containing isoniazid and ethambutol, *Arzneimittelforschung*, **1995**, *45*, 1236–1239.

SAMPLE
Matrix: blood
Sample preparation: 500 μL Serum + 100 μL 30% trichloroacetic acid, mix, centrifuge at 2000 g for 5 min. Remove a 100 μL aliquot of the supernatant and add it to 20 μL 0.1% trans-cinnamaldehyde in MeOH, let stand for 10 min, add 20 μL 1 M KOH, inject an aliquot.

HPLC VARIABLES
Column: 125 × 3.9 4 μm Nova-pak C18
Mobile phase: MeCN:water:triethylamine:acetic acid 40:60:0.2:0.1, pH 5 ± 1
Flow rate: 1.3
Detector: UV 340

CHROMATOGRAM
Retention time: 1.95
Limit of detection: 20 ng/mL

OTHER SUBSTANCES
Noninterfering: acepromethazine, adrafinil, allopurinol, alprostadil, altretamine, atenolol (tenormine), baclofen, bendroflumethiazide, benserazide, betamethasone, bisoprolol, bromocriptine, caffeine, captopril, chlorpromazine, clomipramine, clonazepam, cortisone, cyamemazine, difebarbamate, dothiepin (dosulepin), ethosuximide, fenspiride, flumazenil, fluoxetine, fluvoxamine, halofantrine, hydrochlorothiazide, hydroxyzine, ibuprofen, imipramine, levamisol, levodopa, maprotiline, medifoxamine, metopimazine, midazolam, nafronyl (naftidrofuryl), naftazone, naproxen, nicergoline, nitrazepam, nordazepam, nortriptyline, penfluridol, phenobarbital, pimozide, pipamperone, pipotizine, primidone, pyrazinamide, pyridoxine, quinine, rifampin, selegiline (deprenyl), streptomycin, tetrazepam, theophylline, thioproperazide, tiapride, triazolam, trihexyphenidyl, trimeprazine (alimemazine), trimipramine, tropatepine, vigabatrin, zopiclone

KEY WORDS
serum; derivatization

REFERENCE
Sadeg,N.; Pertat,N.; Dutertre,H.; Dumontet,M. Rapid, specific and sensitive method for isoniazid determination in serum, *J.Chromatogr.B*, **1996**, *675*, 113–117.

SAMPLE
Matrix: blood, CSF
Sample preparation: 200-500 μL Plasma or CSF + 50 μL 50 μg/mL phenelzine sulfate + 100 μL 10% (?) aqueous acetic acid + 5 mL n-hexane, shake for 30 min, centrifuge at 1870 g for 10 min. Discard the organic layer. Add 300 μL 0.1% salicaldehyde in EtOH and 400 μL 10% aqueous acetic acid to the aqueous layer, heat at 60° for 30 min, cool, add 1 mL 1 M pH 6.5 K_2HPO_4, shake for 10 s, add 5 mL diethyl ether, shake for 10 min, centrifuge at 1870 g for 10 min. Remove the organic layer and evaporate it to dryness under a stream of nitrogen at 40°, reconstitute the residue in 50 μL mobile phase, inject a 25 μL aliquot.

HPLC VARIABLES
Guard column: 30 × 4.6 30 μm C8 (Waters)
Column: 300 × 3.9 10 μm μBondapak C18
Mobile phase: MeCN:water:triethylamine 70:30:0.4 containing 5 mM heptanesulfonic acid, pH adjusted to 6.0 with acetic acid
Flow rate: 1
Injection volume: 25
Detector: UV 320

CHROMATOGRAM
Retention time: 1.6
Internal standard: phenelzine sulfate (3)
Limit of detection: 200 ng/mL

OTHER SUBSTANCES
Extracted: hydrazine
Noninterfering: p-aminosalicylic acid, pyrazinamide, rifampin

KEY WORDS
plasma; rabbit; derivatization

REFERENCE
Walubo,A.; Chan,K.; Wong,C.L. Simultaneous assay for isoniazid and hydrazine metabolite in plasma and cerebrospinal fluid in the rabbit, *J.Chromatogr.*, **1991**, *567*, 261–266.

SAMPLE
Matrix: blood, CSF
Sample preparation: 200 μL Serum, plasma, or CSF + 300 μL reagent. Flush column A to waste with 500 μL 500 mM ammonium sulfate, inject sample onto column A, flush column A to waste with 500 μL 500 mM ammonium sulfate, elute the contents of column A onto column B with mobile phase, monitor the effluent from column B. (Reagent was 8.05 M guanidine hydrochloride and 1.02 M ammonium sulfate in water.)

HPLC VARIABLES
Column: A 30 × 2.1 40 μm preparative grade C18 (Analytichem); B 250 × 4.6 10 μm Partisil C8
Mobile phase: Gradient. A was 50 mM pH 4.5 KH_2PO_4. B was MeCN:isopropanol 80:20. A:B 90:10 for 1 min, to 30:70 over 15 min, maintain at 30:70 for 4 min.
Column temperature: 50
Flow rate: 1.5
Detector: UV 280 for 5 min then UV 254

CHROMATOGRAM
Retention time: 3.10

Internal standard: heptanophenone (19.2)
Limit of quantitation: 2500 ng/mL

OTHER SUBSTANCES
Extracted: acetazolamide, ampicillin, bromazepam, caffeine, carbamazepine, chloramphenicol, chlorothiazide, diazepam, droperidol, ethionamide, furosemide, methadone, penicillin G, phenobarbital, phenytoin, prazepam, propoxyphene, pyrazinamide, rifampin, trimeprazine, trimethoprim

KEY WORDS
plasma; serum; column-switching

REFERENCE
Seifart,H.I.; Kruger,P.B.; Parkin,D.P.; van Jaarsveld,P.P.; Donald,P.R. Therapeutic monitoring of antituberculosis drugs by direct in-line extraction on a high-performance liquid chromatography system, *J.Chromatogr.*, **1993**, *619*, 285–290.

SAMPLE
Matrix: blood, CSF, urine
Sample preparation: Centrifuge 1.5 mL whole blood, CSF, or urine at 3000 g for 3 min, add 500 μL of the supernatant to 500 μL 10% trichloroacetic acid, centrifuge at 10000 g for 1 min. Remove a 200 μL aliquot and add it to 20 μL water, add 40 μL 1% trans-cinnamaldehyde in MeOH, let stand at room temperature for 10 min, inject a 20 μL aliquot.

HPLC VARIABLES
Column: 250 × 4.6 Partisil 5 C8
Mobile phase: Gradient. A was 50 mM KH_2PO_4. B was MeCN:isopropanol 80:20. A:B 60:40 for 1 min, to 30:70 over 9 min, maintain at 30:70 for 4.5 min, re-equilibrate at initial conditions for 4 min.
Column temperature: 50
Flow rate: 1
Injection volume: 20
Detector: UV 340

CHROMATOGRAM
Retention time: 7
Limit of detection: 500 ng/mL

OTHER SUBSTANCES
Extracted: hydrazine

KEY WORDS
whole blood; derivatization

REFERENCE
Seifart,H.I.; Gent,W.L.; Parkin,D.P.; van Jaarsveld,P.P.; Donald,P.R. High-performance liquid chromatographic determination of isoniazid, acetylisoniazid and hydrazine in biological fluids, *J.Chromatogr.B*, **1995**, *674*, 269–275.

SAMPLE
Matrix: blood, urine
Sample preparation: Condition a 3 mL CN-bonded maxi-clean cartridge (Alltech) with 5 mL MeOH and 2 mL 1% aqueous acetic acid. 2 mL Plasma or urine + 1 mL isopropanol:chloroform 50:50 + 1 μg rifampin, shake for 30 s, add to the SPE cartridge with rinses, collect the eluate, inject an aliquot of the eluate.

HPLC VARIABLES
Column: 250 × 4.6 5 μm Excalibar C18-CN (Alltech)
Mobile phase: MeOH:5 mM tetra-n-butylammonium hydroxide 80:20 adjusted to pH 3.0 with phosphoric acid
Flow rate: 1.5

Injection volume: 20
Detector: UV 265

CHROMATOGRAM
Retention time: 2.7
Internal standard: rifampin (4.3)
Limit of detection: 250 ng/mL (urine), 200 ng/mL (plasma)

OTHER SUBSTANCES
Extracted: pyrazinamide

KEY WORDS
plasma; SPE

REFERENCE
Gaitonde,C.D.; Pathak,P.V. Rapid liquid chromatographic method for the estimation of isoniazid and pyrazinamide in plasma and urine, *J.Chromatogr.*, **1990**, *532*, 418–423.

SAMPLE
Matrix: milk
Sample preparation: Condition a 3 mL 500 mg C18 SPE cartridge (J.T. Baker) with 6 mL MeOH and 6 mL water. 80 mL milk + 20 mL 20% trichloroacetic acid in water, mix, let stand at room temperature for 5 min, centrifuge at 5500 g for 15 min. Filter (0.45 μm) 75 mL of the liquid, add 1 mL 1% cinnamaldehyde in MeOH to the filtrate, mix for a few s, let stand at room temperature for 15 min, add to the SPE cartridge at 5 mL/min, dry for 10 min, wash with 3 mL mobile phase at 0.2 mL/min, wash with 5 mL n-hexane at 0.3 mL/min, dry for 10 min, elute with 6 mL MeOH. Evaporate the eluate under reduced pressure at 35-40°, reconstitute the residue with 200 μL mobile phase, inject an aliquot.

HPLC VARIABLES
Guard column: 4 × 4 5 μm LiChrospher 100-CN
Column: 250 × 4 5 μm LiChrospher 100-CN
Mobile phase: MeOH:water 40:60 containing 0.41 g/L sodium acetate trihydrate and 10 mL/L glacial acetic acid
Flow rate: 1
Injection volume: 100
Detector: UV 330

CHROMATOGRAM
Retention time: 7.4
Limit of detection: 0.1 ng/mL

KEY WORDS
cow; SPE; derivatization

REFERENCE
Defilippi,A.; Piancone,G.; Costa Laia,R.; Balla,S.; Tibaldi,G.P. High-performance liquid chromatography with UV detection and diode-array UV confirmation of isonicotinic acid hydrazide in cattle milk, *J.Chromatogr.B*, **1994**, *656*, 466–471.

SAMPLE
Matrix: milk
Sample preparation: Condition a 3 mL 500 mg 40 μm phenyl SPE cartridge (J.T. Baker) with 6 mL MeOH and 6 mL water. 80 mL Milk + 20 mL 20% trichloroacetic acid in water, let stand at room temperature for 5 min, centrifuge at 5500 g for 15 min, filter (0.45 μm cellulose acetate) a 75 mL aliquot. Add the filtrate to 1 mL 1% cinnamaldehyde in MeOH, mix for a few s, let stand at room temperature for 15 min. Add 70 mL of the sample to the SPE cartridge at 3 mL/min, wash with 3 mL MeOH:water 40:60 at 3 mL/min, dry under vacuum for 1 min, wash with 3 mL MeOH:water 46:54 at 3 mL/min, dry under vacuum for 1 min, wash with 3 mL n-hexane at 3 mL/min, dry under vacuum for 1 min, elute with 6 mL MeOH. Evaporate the eluate under vacuum, reconstitute in 233 μL mobile phase, inject an aliquot.

HPLC VARIABLES
Guard column: 4 × 4 5 μm LiChrosphere 100 RP18
Column: 250 × 4 5 μm LiChrosphere 100 RP18
Mobile phase: MeCN:MeOH:buffer 41:13:46 (Buffer was 1% ammonium acetate adjusted to pH 5.5 with acetic acid.)
Flow rate: 1
Injection volume: 100
Detector: UV 330

CHROMATOGRAM
Retention time: 4.02
Limit of detection: 0.05 ng/mL

KEY WORDS
cow; SPE; derivatization

REFERENCE
Defilippi,A.; Piancone,G.; Costa Laia,R.; Tibaldi,G.P. An HPLC screening method for the detection of isonicotinic acid hydrazide in cattle milk, *Chromatographia*, **1995**, *40*, 170–174.

SAMPLE
Matrix: reaction mixtures
Sample preparation: Centrifuge, inject a 20 μL aliquot.

HPLC VARIABLES
Column: 250 × 4.6 5 μm Microsorb C8
Mobile phase: MeOH:0.4 g/L $(NH_4)H_2PO_4$ + 0.1% triethylamine (pH 10.0) 10:90
Flow rate: 1
Injection volume: 20
Detector: UV 254

CHROMATOGRAM
Retention time: 5.3
Limit of detection: 10000 ng/mL

REFERENCE
Lunn,G.; Sansone,E.B. Reductive destruction of dacarbazine, procarbazine hydrochloride, isoniazid, and iproniazid, *Am.J.Hosp.Pharm.*, **1987**, *44*, 2519–2524.

SAMPLE
Matrix: solution
Sample preparation: Mix 1 mL pH 7.4 phosphate buffer or hepatocyte solution with 3 mL ethyl acetate:n-butanol 2:1, 300 μL 500 mM tetra-n-butylammonium hydroxide, and 100 μL 80 μg/mL IS in water. Shake the mixture for 20 min, centrifuge at 850 g for 15 min, remove 2.5 mL of the upper organic layer, mix with 1 mL 0.2% hydrobromic acid, shake for 20 min, centrifuge at 850 g for 15 min, discard the upper organic layer, inject a 5 μL aliquot of the aqueous solution.

HPLC VARIABLES
Column: 250 × 4.6 5 μm TSK-gel ODS-80Ts
Mobile phase: MeOH:67 mM KH_2PO_4 4:96
Column temperature: 37
Flow rate: 0.8
Injection volume: 5
Detector: UV 265

CHROMATOGRAM
Retention time: 11.42
Internal standard: 6-methylnicotinic acid (8)
Limit of quantitation: 2 μg/mL

OTHER SUBSTANCES
Simultaneous: acetylisoniazid, isonicotinic acid

KEY WORDS
rat; hepatocytes

REFERENCE
Ono,Y.; Noda,A.; Zaima,Y.; Jitsufuchi,N.; Eto,S.; Noda,H. Determination of isonicotinic acid in the presence of isoniazid and acetylisoniazid. Studies on isonicotinic acid formation from isoniazid in isolated rat hepatocytes, *J.Chromatogr.B*, **1996**, *677*, 339–343.

SAMPLE
Matrix: solutions
Sample preparation: Centrifuge and filter cell solutions (0.22 μm), inject an aliquot.

HPLC VARIABLES
Guard column: Guard-PAK C18 (Waters)
Column: 300 × 3.9 5 μm μBondapak C18
Mobile phase: MeOH:50 mM pH 6.0 KH_2PO_4 3:97
Flow rate: 3
Detector: UV 254

CHROMATOGRAM
Retention time: 3.5

REFERENCE
Koga,H. High-performance liquid chromatography measurement of antimicrobial concentrations in polymorphonuclear leukocytes, *Antimicrob.Agents Chemother.*, **1987**, *31*, 1904–1908.

SAMPLE
Matrix: solutions

HPLC VARIABLES
Column: 250 × 4.6 Zorbax RX
Mobile phase: Gradient. A was 10 mL concentrated orthophosphoric acid and 7 mL triethylamine in 1 L water. B was 10 mL concentrated orthophosphoric acid and 7 mL triethylamine in 200 mL water, make up to 1 L with MeCN. A:B from 100:0 to 0:100 over 30 min, maintain at 0:100 for 5 min.
Column temperature: 30
Flow rate: 2
Detector: UV 210

OTHER SUBSTANCES
Also analyzed: acepromazine, acetaminophen, acetophenazine, albuterol, aminophylline, amitriptyline, amobarbital, amoxapine, amphetamine, amylocaine, antipyrine, aprobarbital, aspirin, atenolol, atropine, avermectin, barbital, benzocaine, benzoic acid, benzotropine, benzphetamine, berberine, bibucaine, bromazepan, brompheniramine, buprenorphine, buspirone, butabarbital, butacaine, butethal, caffeine, carbamazepine, carbromal, chloramphenicol, chlordiazepoxide, chloroquine, chlorothiazide, chloroxylenol, chlorphenesin, chlorpheniramine, chlorpromazine, chlorpropamide, chlortetracycline, cimetidine, cinchonidine, cinchonine, clenbuterol, clonazepam, clonixin, clorazepate, cocaine, codeine, colchicine, cortisone, coumarin, cyclazocine, cyclobenzaprine, cyclothiazide, cyheptamide, cymarin, danazol, danthron, dapsone, debrisoquine, desipramine, dexamethasone, dextromethorphan, dextropropoxyphene, diamorphine, diazepam, diclofenac, diethylpropion, diethylstilbestrol, diflunisal, digitoxin, digoxin, diltiazem, diphenhydramine, diphenoxylate, diprenorphine, dipyrone, disulfiram, dopamine, doxapram, doxepin, dronabinol, ephedrine, epinephrine, epinine, estradiol, estriol, estrone, ethacrynic acid, ethosuximide, etonitazene, etorphine, eugenol, famotidine, fenbendazole, fencamfamine, fenoprofen, fenproporex, fentanyl, flubendazole, flufenamic acid, flunitrazepam, 5-fluorouracil, fluoxymesterone, fluphenazine, furosemide, gentisic acid, gitoxigenin, glipizide, glunixin, glutethimide, glybenclamide, guaiacol, halazepam, haloperidol, hydrochlorothiazide, hydrocodone, hydrocortisone, hydromorphone, hydroxyquinoline, ibogaine, ibuprofen, imino-

stilbene, imipramine, indomethacin, isocarbostyril, isoproterenol, isoxsuprine, ivermectin, ketamine, ketoprofen, kynurenic acid, levorphanol, lidocaine, lorazepam, lormetazepam, loxapine, mazindol, mebendazole, meclizine, meclofenamic acid, medazepam, mefenamic acid, megestrol, mepacrine, meperidine, mephentermine, mephenytoin, mephesin, mephobarbital, mepivacaine, mescaline, mesoridazine, methadone, methamphetamine, methapyrilene, methaqualone, methazolamide, methocarbamol, methoxamine, methsuximide, methyl salicylate, methyldopa, methyldopamine, methylphenidate, methylprednisolone, methyltestosterone, methyprylon, metoprolol, mibolerone, morphine, nadolol, nalorphine, naloxone, naltrexone, naphazoline, naproxen, nefopam, niacinamide, nicotine, niacin, nifedipine, niflumic acid, nitrazepam, norepinephrine, nortriptyline, noscapine, nylidrin, oxazepam, oxycodone, oxymorphone, oxyphenbutazone, oxytetracycline, papaverine, pargyline, pemoline, pentazocine, pentobarbital, persantine, phenacetin, phenazocine, phenazopyridine, phencyclidine, phendimetrazine, phenelzine, pheniramine, phenobarbital, phenothiazine, phensuximide, phentermine, phenylbutazone, phenylephrine, phenylpropanolamine, piperocaine, prazepam, prednisolone, primidone, probenecid, progesterone, propiomazine, propranolol, propylparaben, pseudoephedrine, puromycin, pyrilamine, pyrithyldione, quazepam, quinaldic acid, quinidine, quinine, ranitidine, recinnamine, reserpine, resorcinol, saccharin, albuterol, salicylamide, salicylic acid, scopolamine, scopoletin, secobarbital, strychnine, sulfacetamide, sufadiazine, sulfadimethoxine, sulfaethidole, sulfamerazine, sulfamethazine, sulfamethoxizole, sulfanilamide, sulfapyridine, sulfasoxizole, sulindac, tamoxifen, temazepam, testosterone, tetracaine, tetracycline, tetramisole, thebaine, theobromine, theophylline, thiabendazole, thiamine, thiamylal, thiobarbituric acid, thioridazine, thiosalicylic acid, thiothixene, thymol, tolazamide, tolazoline, tobutamide, tolmetin, tranylcypromine, triamcinolone, tribenzylamine, trichloromethiazide, trifluoperazine, trihexyphenidyl, trimethoprim, tripelennamine, triprolidine, tropacocaine, tyramine, verapamil, vincamine, warfarin, yohimbine, zoxazolamine

REFERENCE

Hill,D.W.; Kind,A.J. Reversed-phase solvent gradient HPLC retention indexes of drugs, *J.Anal.Toxicol.*, **1994**, *18*, 233–242.

SAMPLE
Matrix: solutions

HPLC VARIABLES
Column: 250 × 4.6 12 μm Dynamax C18 (Rainin)
Mobile phase: pH 7.5 sodium phosphate buffer
Flow rate: 2
Detector: UV 266

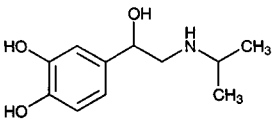
CHROMATOGRAM
Retention time: 5.4

OTHER SUBSTANCES
Simultaneous: metabolites, acetylisoniazid

REFERENCE

Hickman,D.; Palamanda,J.R.; Unadkat,J.D.; Sim,E. Enzyme kinetic properties of human recombinant arylamine N-acetyltransferase 2 allotypic variants expressed in *Escherichia coli*, *Biochem.Pharmacol.*, **1995**, *50*, 697–703.

Isoproterenol



Molecular formula: $C_{11}H_{17}NO_3$
Molecular weight: 211.26
CAS Registry No.: 7683-59-2, 51-30-9 (HCl), 6700-39-6 (sulfate dihydrate), 299-95-6 (sulfate)
Merck Index: 5236

SAMPLE
Matrix: blood